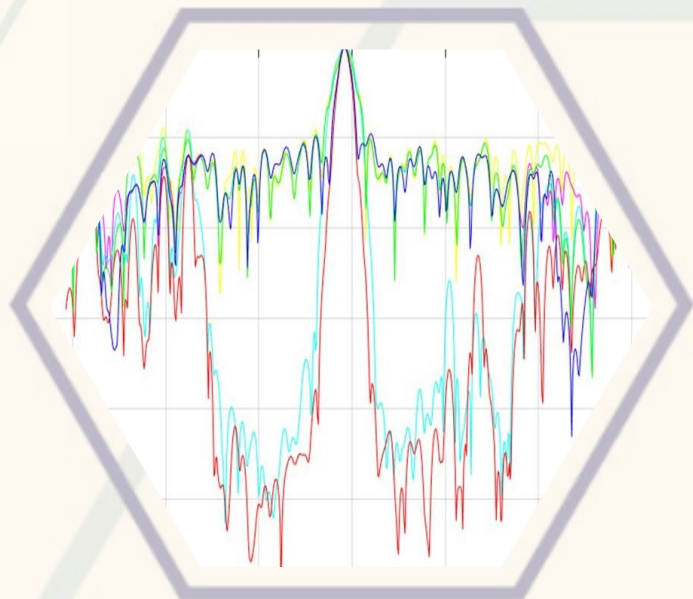
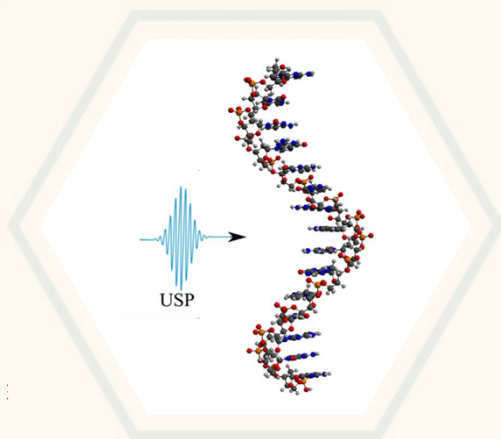


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Visualization and Characterization of Nucleotide Sequences in Bioinformatics: State-of-Art in the Past and Present

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Abstract. The review considers the main trends in the development of the methods for representation and characterization of DNA-associated nucleotide sequences, starting from the key works performed in the nineties of the last century and ending with the current state of the art. The basic principles of algorithms for processing nucleotide sequences and generating representing 2D and 3D objects are discussed in relation to such popular methods as polyline representation (generation of H-, Z-, C-, ... curves), chaos game representation, and sequence logo representation. In addition, novel approaches to representation of genetic information using the principles of coherent optical information processing, such as GB speckle technique and polarization mapping, are considered. The discussion is illustrated by the examples of graphic images obtained using the basic algorithms for model nucleotide sequences or sequences borrowed from open sources. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: nucleotide sequences; graphical imaging; polyline representation; chaos game representation; sequence logo; coherent optical imaging.

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1 Introduction

The growing challenges facing humanity due to pandemic nature of emergence and spread of various pathogenic microorganisms with high variability in the past few decades have led, in particular, to a rapid development of such interdisciplinary field as bioinformatics. This area of modern science combines the latest fundamental and applied achievements of such sciences as biochemistry, biophysics, information theory, mathematical statistics, the theory of random processes, etc. Currently, the procedure for obtaining and processing genetic information about various biological objects includes two essential stages, the first of which is sequencing of the raw biological material using the modern next-generation sequencing (NGS) technology: short-read and long-read sequencing techniques (see,

e.g., [1]). In these technologies, the identification of certain nucleotides or their groups is usually carried out using fluorescent techniques (for instance, applying specific fluorescent labels). In addition, the identification can be based on measurements of ion currents (for example, due to changes in the concentration of hydrogen ions during DNA polymerization when interacting with certain types of deoxyribonucleotide triphosphates); this technique is defined as the ion semiconductor sequencing [1]. As a result, quasi-random sequences of four characters (A, C, T, G) associated with the nitrogenous bases (A – adenine, C – cytosine, T – thymine, G – guanine) that make up DNA are formed. These are heterocyclic organic compounds, which are derivatives of pyrimidine and purine. Adenine ($C_5H_5N_5$) and guanine ($C_5H_5N_5O$) are purine derivatives, while cytosine ($C_4H_5N_3O$) and thymine ($C_5H_6N_2O_2$) are

pyrimidine derivatives. Between the nitrogenous bases of deoxyribonucleotides of two different chains, three or two hydrogen bonds are formed (guanine binds to cytosine with three bonds, and adenine with thymine with two) [2].

The length of sequencing-recovered fragments, beginning from the so-called start codons, can vary from several hundred to tens of thousands letters. Protein synthesis occurs in ribosomes, and information from a DNA molecule is transferred by an RNA molecule, which is an intermediary. The RNA polymerase enzyme moves along the DNA chain and, falling on the ATG triplet (the start codon), initiates the beginning of the synthesis of the RNA molecule. The synthesis continues until the RNA polymerase occurs at the stop codon [2].

Identification, analysis, and visualization of these sequence fragments are the main tasks of modern bioinformatics, related to the second essential stage of genetic information processing. Over the past thirty years, abundance of various approaches has been developed to solve the problems of analyzing and visualizing symbol sets associated with sequenced nucleotide sequences. The key principles of these approaches differ significantly and are based on a variety of mathematical methods and algorithms, ranging from information entropy estimates according to Kolmogorov and Shannon to application of the concepts of the chaos game theory. In the last few years, some works related to the possible use of coherent light fields as carriers and converters of specially encoded genetic information have been published.

The purpose of this review is to consider the current state of the art of the methods for analyzing and visualizing genetic information presented in the form of symbolic sequences, to systematize these methods, and to discuss their advantages and disadvantages.

2 Inherent Statistical Properties of Nucleotide-Associated Symbol Sequences

Despite a seemingly random nature of the sequencing-recovered symbol series, they are characterized by a certain statistical heterogeneity. At the lower level, corresponding to individual symbols, this heterogeneity manifests itself in different relative frequencies of manifestations of each of the 4 symbols during sequential reading of the letter series. As an example, Fig. 1 shows relative frequencies of manifestation of adenine, cytosine, thymine, and guanine in nucleotide sequences corresponding to three pathogenic objects: the model Spike gene of the SARS-CoV-2 virus [3] Wuhan strain (the GenBank Acc. No. EPI_ISL_402124) [4], Influenza A virus (A/Weiss/43 (H1N1)) neuraminidase (NA) gene, complete cds GenBank: AF250365.2 [5], and Variola virus, complete genome NCBI Reference Sequence: NC_001611.1 [6].

At a higher level, corresponding to combination of individual nucleotide-associated symbols into groups of a given length, more flexible and versatile possibilities open up for identifying and analyzing nucleotide

sequences. Traditionally, the sequences are divided into groups of three characters (triplets), beginning from the start codon (e.g., ATA, CTG, AAT, CAC, etc.). Thus, the number of different triplets is determined by a combinatorial evaluation of a number of possible combinations of four objects in groups of three items, which is 64.

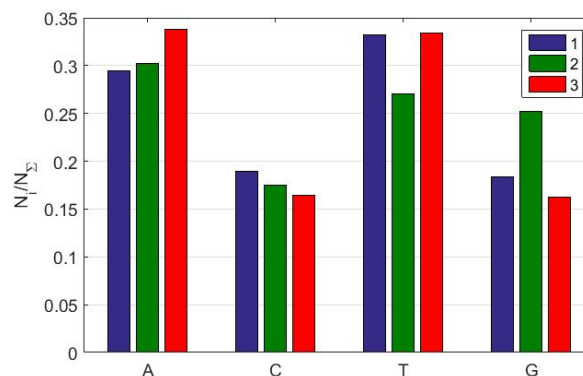


Fig. 1 The relative frequencies of appearance of four base nucleotides in DNA sequences of three pathogenic objects: 1 – Wuhan strain of the SARS-CoV-2 virus; 2 – Influenza A/H1N1 virus; 3 – Variola virus.

The hypothesis that DNA-associated nucleotide series have fractal properties was put forward in the nineties of the past century by a group of researchers [7] from the Institute of Molecular Genetics and Institute of Chemical Physics of the Russian Academy of Sciences. These properties are associated with existence of long-range power-law correlations along the DNA sequences established by a number of researchers almost at the same time [8–13]. In particular, a characteristic manifestation of such long-range correlations is the deviation of the exponent α of the so-called Peng function [8] $P(l) \propto l^\alpha$ from the trivial value of 0.5 towards larger values (of the order of 0.6–0.7). The Peng function is introduced as the r.m.s. value of the purine (A,G) – pyrimidine (C,T) difference in sequence fragments with the length of l depending on l .

Consideration of the fractal properties of nucleotide sequences through the estimates of the so-called Hurst exponent or a set of critical exponents can be classified as one of the approaches to a generalized description of inherent features of these sequences. As another generalized approach, we can note the definitions of complexity measures carried out using various techniques. Generally speaking, the idea of evaluating complexity of the texts goes back to the fundamentals of the information theory and was particularly discussed by Kolmogorov [14]. In 1976, J. Ziv and A. Lempel proposed an efficient technique for estimating the complexity measure of symbolic sequences of the finite length [15], which in various modifications has been applied to bioinformatics problems (in particular, for the compression of genetic information) [16–18]. In the Ziv-Lempel method, the complexity measure of a given symbol sequence is evaluated as a minimal number of

steps necessary for the sequence synthesis in the framework of a certain procedure. This procedure allows for two operations: generation of a new symbol or copying a fragment from an already synthesized sequence.

In addition to the Ziv-Lempel complexity measure, the measure of linguistic complexity is also applied in bioinformatics [19, 20]. This parameter of one-dimensional symbol sequences is introduced in terms of vocabulary usage. This is the ratio of actual vocabulary of words (symbol combinations) of a given length L_w to the maximal possible vocabulary for the given sequence. The linguistic complexity a product of all vocabulary usages for $1 \leq L_w \leq N_t - 1$ (N_t is the number of symbols in the sequence).

In addition to the integral estimates of complexity and entropy [21, 22] of quasi-random symbol strings obtained by sequencing, other statistical approaches have been successfully applied in the generalized analysis of the properties of nucleotide series. These approaches include, for example, the method of the Bayes prediction [23], features of the Markov [24, 25] or Jukes-Cantor [26] processes, the Monte-Carlo method [27], specially designed probabilistic models [28], and multidimensional scaling and clustering techniques [29, 30].

3 Polyline Representation of Nucleotide Sequences in 2D and 3D Spaces

The eighties and nineties of the past century as well as the 2000s were marked by emergence of a large number of approaches to a polyline-based 2D- or 3D-visualization of DNA-associated symbol sequences. A variety of approaches that display distributions of the basic nucleotides in the analyzed fragments of the sequenced DNA in various ways in the plane and in space, gave rise to the concepts of imaging curves of the H-type, Z-type, etc. In general, the noticeable differences between these imaging techniques come down to two main points:

- the choice of the initial basis for 2D or 3D display, based on existence of only four basic nucleotides (A, C, T, G) and with account to the differences between them in terms of membership in the pyrimidine or purine groups, amino or keto groups, as well as in strength of the hydrogen bonds (weak or strong);

- the algorithm of an iterative procedure used to generate the subsequent start-to-end coordinates of the line segments in 2D or 3D space at a given iteration step.

Certainly, one of the pioneering works in this area was carried out by E. Hamori and J. Riskin in 1983 [31]. In fact, they established the general principles for specifying the bases for dot or polyline imaging of nucleotide sequences, which were then applied and modified in a number of further works. The Hamori-Riskin displaying technique was defined as a synthesis of the H-curve, which gradually falls down along the z-direction in the 3D Cartesian space, depending on the current position of a displayed nucleotide in the

sequence. This current position is associated with a descending z-coordinate, and the (A, C, T, G) basis is localized on the plane $(x, y, z = 0)$. A visualized nucleotide at the position z is associated with a specific vector $\bar{g}(z)$ introduced following the rule:

$$A \rightarrow \bar{g}(z) = \bar{i} + \bar{j} - \bar{k} ; \quad C \rightarrow \bar{g}(z) = -\bar{i} - \bar{j} - \bar{k} ; \\ T \rightarrow \bar{g}(z) = \bar{i} - \bar{j} - \bar{k} ; \quad G \rightarrow \bar{g}(z) = -\bar{i} + \bar{j} - \bar{k} .$$

Accordingly, the resulting vector $\bar{h}(n)$, which defines the end of a line segment belonging to the H-curve at the n -position, is determined by summation of the $\bar{g}(z)$

vectors: $\bar{h}(n) = \sum_{z=1}^n \bar{g}(z)$. Following Hamori and Riskin,

the H-curve “will be a space curve consisting of a series of n joined base vectors assembled in a head-to-tail fashion in the $-z$ direction” [31].

Based on the algorithm for generating the H-curve, it can be concluded that the y-projection of the vector $\bar{h}(n)$ characterizes excess in the number of purine nucleotides in the analyzed sequence over the pyrimidine nucleotides. In a similar way, the x-component indicates the dominance of amino nucleotides over the keto ones (note that these dominances can be both positive and negative). Among the advantages of the considered H-curve technique, the authors highlighted a possibility for identifying specific features of the visualized nucleotide sequences (in particular, preponderance of purine nucleotides, richness of A and T nucleotides, presence of only pyrimidine nucleotides, etc.) by visual inspection of the generated H-curve.

In the decade since the publication of Hamori and Riskin's work, several approaches to the polyline 2D-visualization of nucleotide sequences have been considered [32–40]. All of them are based on the general principle of a step-by-step generation of a displaying polyline along the rendered sequence in (A, C, T, G)-assigned coordinates. However, the rules for finding the start and end coordinates for a line segment at a given step can slightly differ from one approach to another. In particular, Nandy [33] used a segment generation rule in 2D space that is in general similar to the Hamori & Riskin's algorithm for the 3D imaging. Leong and Morgenthaker [34] considered a random walk presentation for DNA sequences on the (A, C, T, G) plane. In this approach, a step-by-step generation of the imaging curve is controlled by the following simple rule: if a displayed nucleotide is A, then the corresponding segment of the unit length is directed to the right; in the case of C it is directed to the left. Similarly, occurrence of T causes the up direction, and G corresponds to the down shift. Accordingly, the line that mimics a random walk trajectory is obtained (as an example, see Fig. 2).

Further development of this methodology in relation to the polyline 3D-imaging of DNA-associated symbol sequences was carried out in Refs. [35–37].

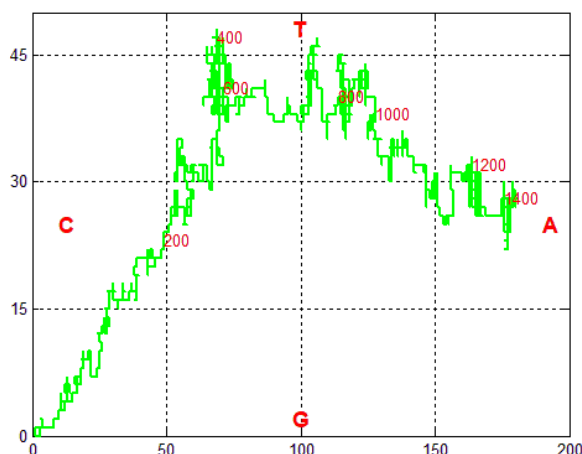


Fig. 2 Example of the random walk representation of the symbol sequence associated with the Influenza A/H1N1 virus.

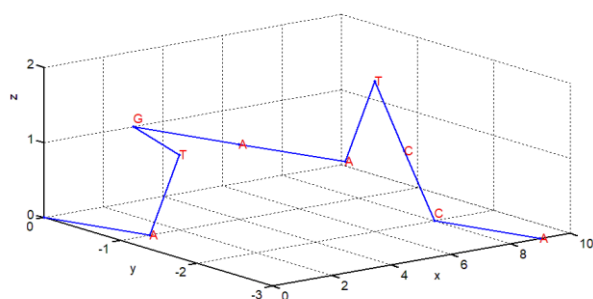


Fig. 3 Example of the recovered initial fragment of the RY-curve for the symbol sequence associated with the Influenza A/H1N1 virus.

However, despite certain advantages of these imaging techniques (in particular, obviousness), they all have a common drawback associated with information losses due to a possible overlapping and degeneracy of the displaying curve [38]. Generation of RY-, MK-, and WS-curves in 3D space, which is free of these disadvantages, was considered in Ref. [38]. In this case, the base points for a pair of nucleotides that are similar in one of three basic features (pyrimidine/purine, amine/keto, or weak hydrogen bond/strong hydrogen bond) are located on one of the coordinate planes (for example, xy), and for the other pair – on a perpendicular plane (e.g., xz). Similar to other polyline techniques, the end coordinates of the displaying segments associated with (A, C, T, G) symbols are generated sequentially using a recursive procedure when moving from a previous position in the symbol series to the given element. A recursive procedure considered in Ref. [38] is based on summing the end coordinates of symbol-associated basic points, following from the order of symbols in the displayed sequence. As a result, a polyline interpreted as the RY-, MK-, or WS-curve occurs in the 3D space. The starting point for all three curves is the origin.

As an example, let us consider the procedure of 3D visualization of the sequence fragment

ATGAATCCA (the initial 9-base fragment of the symbol sequence for the Influenza A/H1N1 virus, [5]) in the RY basis (separation of nucleotides according to the “pyrimidine/purine” feature). In this case, the set of base points in 3D Cartesian coordinates is defined as: $A \rightarrow (1, -1, 0)$, $G \rightarrow (1, 1, 0)$, $T \rightarrow (1, 0, 1)$, $C \rightarrow (1, 0, -1)$; thus, the base points for purines (A, G) are located in the xy -plane, whereas the points corresponding to the pyrimidines lie in the xz -plane. Sequential generation of the nodal points of the RY-curve for a fragment of the model sequence is as follows:

$$\left\{ \begin{array}{l} \text{origin} \rightarrow A \rightarrow T \rightarrow G \rightarrow \\ (0,0,0) \rightarrow (1,-1,0) \rightarrow (2,-1,1) \rightarrow (3,0,1) \rightarrow \\ A \rightarrow A \rightarrow T \rightarrow \\ (4,-1,1) \rightarrow (5,-2,1) \rightarrow (6,-2,2) \rightarrow \\ C \rightarrow C \rightarrow A \rightarrow \\ (7,-2,1) \rightarrow (8,-2,0) \rightarrow (9,-3,0) \end{array} \right\}, \quad (1)$$

and the corresponding fragment of the RY-curve is displayed in Fig. 3.

The MK-curve and the WS-curve can be recovered in a similar way. In the first case, the basis is created as: $A \rightarrow (1, -1, 0)$, $C \rightarrow (1, 1, 0)$, $G \rightarrow (1, 0, 1)$, $T \rightarrow (1, 0, -1)$. Accounting for the strength of the hydrogen bonds (the WS-curve) corresponds to the following basis: $A \rightarrow (1, -1, 0)$, $T \rightarrow (1, 1, 0)$, $G \rightarrow (1, 0, 1)$, $C \rightarrow (1, 0, -1)$. Xie and Mo [38] substantiated such inherent properties of the RY-, MK-, and WS-curves as the absence of loop formation and degeneration, as well as a one-to-one correspondence between the visualized nucleotide sequence and the curves. In addition, the x -coordinate of the end node for all the curves defines the length of a visualized nucleotide sequence. One of important features of all the three curves is that analysis of the coordinates of their projections on the xy - and xz -planes makes it possible to determine the relative frequencies of occurrence of base nucleotides in the visualized sequences (i.e., has clear biological implication).

Among the discussed variety of polyline representations of nucleotide sequences, it is also necessary to note the technique for generating C-curves, proposed in Refs. [39, 40]. Generation of polyline node coordinates in this case is performed using a much more sophisticated procedure compared to those discussed above. On the (x, y) plane, a set of 64 base points is formed with the coordinates $x = (-4; -3; -2; -1; 1, 2, 3, 4)$; $y = (-4; -3; -2; -1; 1, 2, 3, 4)$. Each base point is associated with a certain triplet of nucleotides in accordance with the following scheme: 16 triplets located in the first quadrant of the (x, y) plane contain adenine (A) as the first nucleotide. Accordingly, guanine (G) is the first nucleotide for the triplets of the second quadrant, cytosine (C) for the triplets of the third quadrant, and thymine (T) for the triplets of the fourth quadrant. In each

triplet, the remaining nucleotides are distributed according to the following scheme:

$$\begin{array}{cccc} IGC & IGG & IAG & IAC \\ IGT & IGA & IAA & IAT \\ ICT & ICA & ITA & ITT \\ ICC & ICG & ITG & ITC \end{array}, \quad (2)$$

where I denotes the first nucleotide. For example, the triplet TAT corresponds to the point $(4, -2)$ in the (x, y) plane, and the triplet CGA is assigned to the point $(-3, -2)$. Using this base, sequential generation of the coordinates of the C-polyline nodes is carried out as:

$$\begin{cases} x_n = \sum_{i=1}^n x_i; \\ y_n = \sum_{i=1}^n y_i; \\ z_n = \sum_{i=1}^n x_i y_i, \end{cases} \quad (3)$$

where n is the current position of a displayed nucleotide in the sequence. According to Jafarzadeh and Iranmanesh [40], there are no manifestations of looping and degeneration in the C-polylines synthesized in this way. However, the declared advantages of this method in comparison with other 3D polyline representation techniques are not obvious.

4 The Concept of a Chaos Game Representation (CGR) and its Application in Bioinformatics

Application of the chaos game representation (CGR) in bioinformatics started with the pioneering work of Jeffrey [41], who extended the principles of generating two-dimensional fractal objects to the DNA analysis.

The basic idea of the chaos game representation is to sequentially fill a square area with the dots associated with sequentially located symbols in the mapped DNA-related symbol series. The vertices of the square are related to the (A, C, T, G) symbols in a certain way, and the choice of this relationship is determined by setting the representation configuration. As a rule, three basic configurations are applied, similarly to those used for polyline visualization in 2D and 3D spaces. Thus, if we take the upper left vertex of the square as the starting point and arrange the characters clockwise: CGTA (the RY configuration), GCTA (the MK configuration), and CTGA (the WS configuration). These designations are associated with the symbols on the main and minor diagonals of the square. For example, the RY configuration corresponds to the purines ($R = \{A, G\}$) on the minor diagonal, while the pyrimidines ($Y = \{C, T\}$) are on the main diagonal of the square. The MK

configuration takes into account the correspondence of the basic nucleotides to the amino ($M = \{A, C\}$, the minor diagonal) or keto ($K = \{G, T\}$, the main diagonal) groups. Finally, the WS configuration corresponds to the nucleotides of weak hydrogen bonds $W = \{A, T\}$ at the minor diagonal, and of strong bonds $S = \{C, G\}$ at the main diagonal.

Mapping of the symbol sequence is carried out in accordance with the following recursive procedure (Fig. 4): the first symbol in the sequence is marked by the dot at the midpoint between the square center and the vertex denoted by the same symbol. The dot position corresponding to the second symbol is obtained by placing the dot at the midpoint between the position of the first dot and the vertex square corresponding to the dotted symbol. This procedure is repeated until the end of the mapped sequence. The choice of the base configuration (RY, MK, or WS) drastically affects the recovered CGR maps. A remarkable feature is the expressed large-scale inhomogeneities of the dot density across the map areas; this feature is directly related to the above mentioned existence of long-range correlations along the nucleotide sequences. Note that pure random distributions of A, C, T, G letters in the sequences are displayed by uniform distributions of the dots across the synthesized CGR maps.

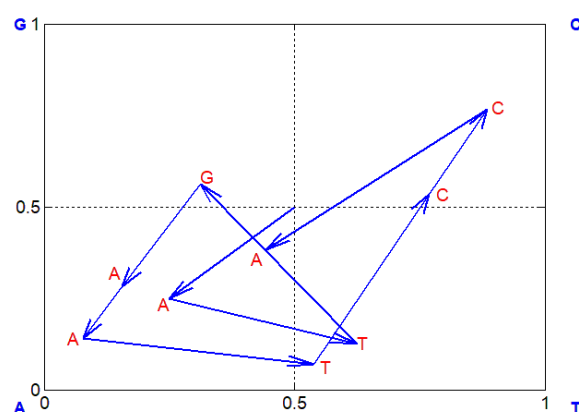


Fig. 4 Example of the initial stage of the CGR map generation in the MK basis for the symbol sequence associated with the Influenza A/H1N1 virus.

A common problem in the synthesis of the CGR maps stems from the limited lengths of the symbol sequences available from DNA sequencing. In other words, the low filling density of the synthesized maps with nucleotide-representing dots reduces reliability of detecting global and local features in distributions of nucleotides along sequenced sequences. As an example, Fig. 5 shows the synthesized maps in the MK base for two symbol sequences corresponding to the available DNA data for the Wuhan strain of the SARS-CoV-2 virus [3] (a) and the Variola virus [6] (b). In the first case, the length of the available sequence was 3822 symbols, while in the second case, 185578 symbols were processed.

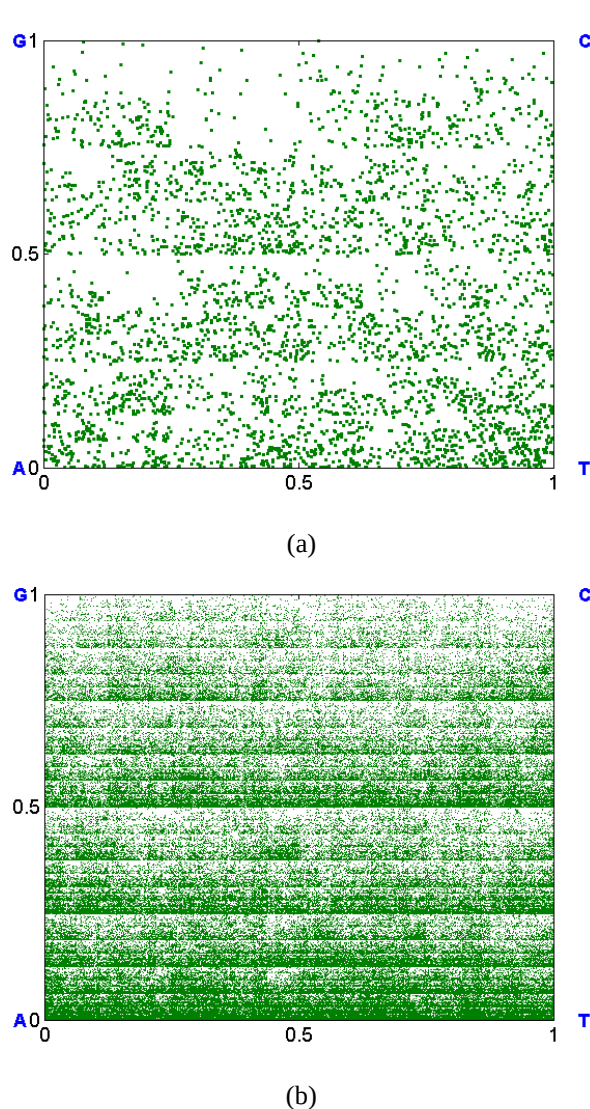


Fig. 5 The synthesized CGR maps for symbol sequences associated with (a) the Wuhan strain of the SARS-CoV-2 virus (3822 bases are available) and (b) the Variola virus (185578 bases are available).

Obviously, the level of identification of significant details on the synthesized chaos game maps dramatically falls down with a decreasing length of the available sequence.

Further development of the CGR technique led to appearance of a frequency chaos game representation (FCGR), in which the problem of small lengths of the analyzed sequences is partially solved. In the FCGR technique, which can be interpreted as a “coarse-grained CGR”, the display square is divided into $N \times N$ sub-squares and the number of imaging dots falling within each square is counted. Thus, the set of 8×8 sub-squares corresponds to the selection of 3-mers (i.e., triplets). Visualization of the obtained FCGR data can be done, for example, using the grayscale coding. As an example, Fig. 6 displays the recovered FCGR grayscale map in the MK base for the Wuhan strain of the SARS-CoV-2 virus (see the left panel in Fig. 5 for comparison).

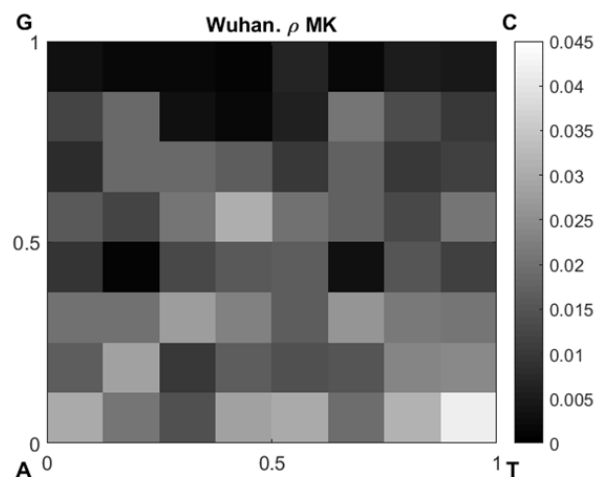


Fig. 6 The (8×8) FCGR grayscale map in the MK base for the Wuhan strain of the SARS-CoV-2 virus.

The basic principles of the FCGR technique were developed by Burma et al. [42]; further progress in this direction was achieved in the works of Huynen et al. ([43], analysis of the GC content in histones), Hill et al. ([44], frequency analysis of dinucleotides for human globin and alcohol dehydrogenase genes). Almeida et al. [45] proposed a more sophisticated way of dividing the imaging square into sub-squares (quadrants) based on the logarithmic relationship between the number q of the quadrants and the number k of nucleotides in the groups: $k = (\log_2(q))/2$. This approach obviously leads to a non-integer resolution of the FCGR mapping. However, the authors justified the use of this logarithmic scaling for addressing redundancy in the genomic structures, which is a major feature, e.g., in the case of amino acid encoding.

Typically, the FCGR maps are plotted in 2D using the gray-scale or color coding; however, it is necessary to note attempts to extend this approach to the 3D space (see, e.g., [46–49]). In particular, in the synthesized 3D CGR maps, the numbers of k -mers are represented along the z -axis instead of a gray scale.

In recent years, various modifications of the CGR method have been actively used to analyze variability of the SARS-CoV-2 virus. In particular, several groups applied the CGR and FCGR techniques to recover phylogenetic trees of coronaviruses [50–52]. Coupling of CGR with an artificial neural network provided additional opportunities to represent the genome and to classify intra-coronavirus sequences [53]. An original approach to the analysis of DNA sequences based on a combination of CGR and modeling of small-angle light scattering is considered in Refs. [54, 55].

5 Sequence Logo Representation

Among a variety of techniques for 2D representation of nucleotide sequences actively used now in bioinformatics, it is necessary to mention the sequence

logo technique introduced by T. D. Schneider and R. M. Stephens in 1990 [56]. This technique aims to identify the most conserved bases across the collections of aligned nucleotide sequences and is based on the estimates of the information entropy following C. Shannon. A sequence logo can be viewed as a specific histogram for a set of nucleotide sequences of the same biological object; this histogram is created according to the following procedure:

- at the first step, the analyzed sequences are aligned with respect to each other;
- next, the frequency sets of occurrences of each nucleotide at each position in the associated sequences are generated;
- with the frequency sets generated, the amount of information (in bits per position) for each position i along the aligned sequences can be defined as:

$$R(i) = 2 + \sum_{m=A}^G f(m,i) \log_2 [f(m,i)] - e(n), \quad (4)$$

- where $f(m,i)$ is the frequency of occurrence of the nucleotide m ($m = A, C, T, G$) at the given position, the second term on the right side is the measure of information uncertainty (entropy) according to Shannon, and the third term is a correction factor that takes into account the number of nucleotide sequences in the analyzed collection;

- contribution of a given symbol to the content of information at a given position is estimated as $f(m,i) \cdot R(i)$.

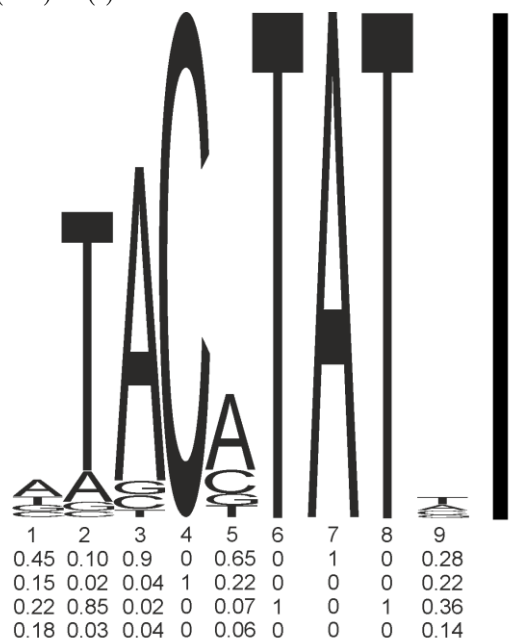


Fig. 7 The fragment of synthesized sequence logo for a model set of the aligned (A, C, T, G) series; the relative frequencies for (A, C, T, G) symbols at each position are presented below the logo; the maximal information content in the stacks (2 bits) is marked by the vertical black bar.

In the sequence logo technique, representation is carried out by setting the height $h(m,i)$ of the visualized symbols at the given position i in accordance with the expression $h(m,i) = f(m,i) \cdot R(i)$. Then the symbols are stacked on top of each other in an increasing order of their frequencies and plotted; this procedure is illustrated in Fig. 7.

6 Visualization and Analysis of Gene-Based Structures Using the Principles of Coherent Optics

The idea of coherent-optical methods for the analysis and visualization of genetic information goes back to the principles of optical information processing. These principles proceed from a unique correspondence between the amplitude-phase characteristics of the coherent light field diffracted by some object and structural properties of an object. Accordingly, by forming some 2D object that in a certain way imitates the structure of the analyzed 1D nucleotide sequence, we can analyze the diffracted light field as a fingerprint of the sequence structure. The GB speckle technology [57] uses this principle and is based on the idea of converting linear A, C, T, G sequences into two-dimensional quasi-random phase matrices read by a coherent light beam. As a result, a speckle pattern is formed in the far diffraction zone with spatial distributions of the amplitude, phase, and intensity that uniquely correspond to distribution of the symbols in the original sequence (abbreviation “GB” corresponds to the term “gene-based”). In particular, the following algorithm was proposed for converting the triplet-divided symbol sequences into two-dimensional phase matrices:

- 1) each triplet is assigned a certain number in the range from 0 to 63 in accordance with the following rule:

$$D_T = 16a_3 + 4a_2 + a_1 - 21, \quad (5)$$

where the lower indices in the right side define the symbol positions in the triplet and the following associations are accepted: $A \rightarrow a = 1$; $C \rightarrow a = 2$, $T \rightarrow a = 3$, $G \rightarrow a = 4$. Accordingly, the zero number corresponds to the combination AAA in the triplet, and the value of 63 is assigned to the GGG triplet;

- 2) from the obtained numeral sequence, a fragment of length N is selected that satisfies the condition $N = n_{\max}^2$, where $n_{\max}^2$ is the maximum square of an integer not exceeding the number of triplets in the analyzed sequence; then this fragment is divided into n sub-fragments of n -th length which are considered as consecutive rows of the matrix $\{a_{ij}\}$ being the basis for the GB phase-modulating matrix $\{\Delta\varphi_{ij}\} = K_\varphi \{a_{ij}\}$, where K_φ is the phase-modulating factor and $a_{ij} = D_T$ for a given triplet;

3) when reading the phase-modulating matrix $\{\Delta\varphi_{ij}\}$ by a collimated laser beam, the GB speckle structure is formed in the far diffraction zone (for example, in the focal plane of the Fourier transform lens).

The choice of the K_φ value is determined by the condition for the formation of a developed speckle pattern [58] in the far diffraction zone. This condition corresponds to the fact that the variance of phase fluctuations in the boundary light field (directly behind the synthesized phase screen) must significantly exceed π^2 . Accordingly, for the case under discussion (the values of D_r are in the range from 0 to 63), the value of the phase modulation factor should significantly exceed $\pi/63$. The developed speckle pattern formed in this case is characterized by a zero mean value of the complex amplitude and negative exponential statistics of intensity fluctuations [58].

As discussed in Refs. [59, 60], GB speckle patterns created using laser radiation, or their digital representations, can be analyzed using coherent optical methods of information processing. However, it should be noted that numerical modeling of decorrelation of complex amplitude spatial distributions in the GB speckle fields at small nucleotide substitutions in the initial sequences shows that such decorrelation is not very significant. Accordingly, the use of GB speckle technology for identification and characterization of mutational changes in the DNA structure is currently rather debatable. In this regard, another approach to coherent-optical visualization and analysis of DNA-associated symbol sequences is of particular interest. This approach is based on polarization coding of the triplets in the analyzed sequences and formation of binary maps of extreme local polarization states in the diffraction zone, which are uniquely associated with the analyzed gene structures [59, 60].

The idea of polarization coding of the triplets is to represent each triplet in the analyzed sequence with the (2×2) submatrix $\{a_{ij}\}$, where each element is associated with one of the four nucleotides (A, C, T, G), and the value of the element corresponds to the content of this nucleotide in the triplet. For example, the triplet ATA is represented as:

$$ATA \rightarrow \begin{pmatrix} 2 & 0 \\ 1 & 0 \end{pmatrix}. \quad (6)$$

Here the following associations are used: $a_{11} \leftrightarrow A$; $a_{12} \leftrightarrow C$; $a_{21} \leftrightarrow T$; $a_{22} \leftrightarrow G$. An inherent property of the submatrices is that the sum of all elements is always 3. After this transformation, a square phase-modulating matrix $\{\tilde{a}_{km}\}$ is assembled from the resulting set of submatrices by combining submatrices line by line. In contrast to the $(N \times N)$ -sized phase-modulating matrices synthesized within the framework of the GB

speckle technique, the $\{\tilde{a}_{km}\}$ matrices used in polarization mapping have the size $(2N \times 2N)$. Another difference lies in a significantly lower bit depth of polarization coding in comparison with the GB speckle technique.

In the modification of the polarization mapping technique considered in Ref. [60], information is read from the matrix $\{\tilde{a}_{km}\}$ by a linearly polarized collimated laser beam with the polarization plane oriented along the main diagonal of the matrix. The phase modulating factor for orthogonally polarized components of the readout beam is set to π ; in addition, for one of the components of the readout beam, the phase shift of $\pi/2$ is introduced with respect to the other orthogonally polarized component. Thus, a quasi-random low-bit set of local polarization states is formed directly behind the phase-modulating matrix over the cross section of the transmitted readout beam.

Note that the mentioned algorithm of polarization modulation of the readout light beam (the phase-modulating factor of π and the phase shift of $\pi/2$ for one of the components) makes it possible to provide a set of close-to-circular local polarization states in the readout plane. As found, this set is very sensitive to local changes in the structure of the encoded nucleotide sequences. In Ref. [60], a slightly different polarization modulation algorithm with reading of local polarization states in the paraxial region of the readout plane is also considered, which allows performing frequency analysis of the nucleotide content in encoded sequences.

The polarization-dependent diffraction pattern corresponding to the analyzed symbol sequence is analyzed in the focal plane of the Fourier-transforming lens in terms of the Stokes vector components $\{s_{t,p}^r\}$.

The $\{s_{p,t}^r\}$ values in the (p,t) point of the readout plane are determined by the amplitudes of x- and y- polarized components and the phase shift between them in the considered point. Thus, the local value of the first component of the Stokes vector ($r=0$) defines the total intensity of the diffracted light in the (p,t) point; the

$\{s_{p,t}^1\}$ value corresponds to the difference of intensities of the x- and y- components in the observation point. The third component $\{s_{p,t}^2\}$ also characterizes the difference in intensities, but in the 45° -rotated coordinate system. Finally, the fourth component $\{s_{p,t}^3\}$ characterizes a contribution of the circular polarization (right or left, depending on the sign) to the total polarization state at the detection point. For convenience of the analysis, it is advisable to consider the normalized values $\{s_{p,t}^{1,2,3}\} = \{s_{p,t}^{1,2,3}/S_{p,t}^0\}$. The possible values of $\{s_{p,t}^{1,2,3}\}$ are in the range from -1 to 1 , and proximity of these values to ± 1 corresponds to the polarization close to the extreme state at the detection point. Accordingly, the binarized maps of distributions of extreme local polarization states

in the readout plane can be considered as unique two-dimensional identifiers of the analyzed symbol sequences. As an example, Fig. 8 (a, b) displays the binarized distributions of $s_{p,t}^3$ extreme states ($-0.999 > s_{p,t}^3 \geq -1$), which correspond to the symbol sequences for the Wuhan and Omicron [61] strains of the SARS-CoV-2 virus. Fig. 8(c) displays the result of the pixel-by-pixel conjunction of Fig. 8(a) and Fig. 8(b) as a representation of differences between these binary maps.

To quantify the differences in the binary distributions of extreme polarization states, the correlation coefficient $R_{1,2}$ can be introduced as $R_{1,2}^{1,2,3} = \sum_{p,t} \xi_{(p,t),1}^{1,2,3} \times \xi_{(p,t),2}^{1,2,3} / \sqrt{\sum_{p,t} \xi_{(p,t),1}^{1,2,3} \times \sum_{p,t} \xi_{(p,t),2}^{1,2,3}}$. Here, the upper index defines the type of extreme polarization state used for analysis, and the lower indices (1, 2) correspond to the reference and analyzed binary patterns. Statistical modeling has shown that the correlation coefficient introduced in this way is highly sensitive to small

measurements in the structure of the analyzed sequence relative to the reference sequence (with the number of nucleotide substitutions of the order of 1–3). As an example, Fig. 9 shows dependence of $R_{1,2}^3$ on the number of substitutions when the symbol sequence for the Wuhan strain of the SarsCov2 virus is applied as a reference. Also, the correlation coefficient for the pairs “Wuhan strain/Omicron strain” and “Wuhan strain/Delta strain” [62] are displayed by the square and triangular markers.

It should be noted that possibilities of the method of polarization mapping of DNA-associated symbol sequences are not limited to the considered algorithm of polarization encoding of initial sequences, as well as to visualization and analysis of distributions of extreme polarization states in the readout plane. In particular, it is possible to provide the frequency analysis of the sequences using the multistage polarization modulation of a readout laser beam by the synthesized phase-modulating matrix $\{\tilde{a}_{km}\}$ [60].

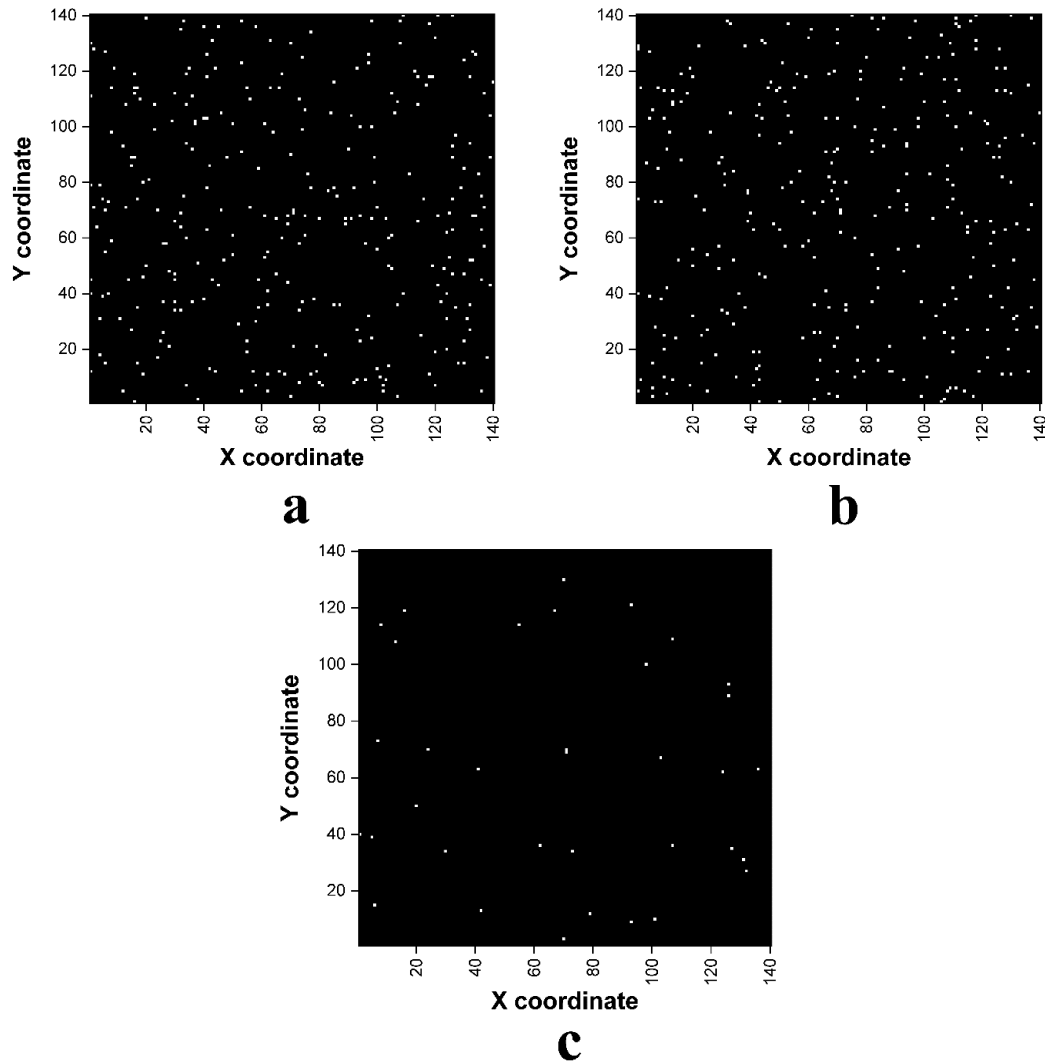


Fig. 8 Binary maps of the extreme polarization state (the predominating left circular polarization with $-0.999 > s_{p,t}^3 \geq -1$) in the cases of (a) Wuhan and (b) Omicron strains of the SARS-CoV-2 virus. So high threshold level (-0.999) was chosen to highlight differences between two binary maps. Panel (c) is the result of the pixel-by-pixel conjunction of panels (a) and (b).

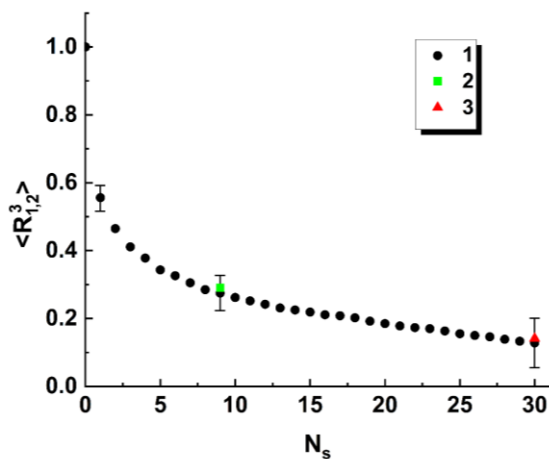


Fig. 9 The correlation between the reference and changed binary distributions $\hat{s}_{(p,t)}^3$ depending on the number of symbol substitutions to the reference (A, C, T, G) sequence (Wuhan strain of the SARS-CoV-2 virus). The threshold level is equal to -0.99 . (1) model data; (2) “Wuhan/Delta” correlation; (3) “Wuhan/Omicron” correlation. Selectively shown error bars correspond to the confidence level of 0.9.

7 Conclusions

Thus, abundance of the methods for graphical representation of nucleotide sequences developed in bioinformatics over the past three decades makes it possible to analyze various features of the genetic structure of biological objects, based on the results of the sequencing DNA fragments. At the same time, it should be noted that none of these methods has sufficient universality in terms of characterization of the genetic structure, which would make it possible to displace other approaches from the field of practical applications in bioinformatics. Judging by the number of references, one of the most popular methods is the chaos game representation (CGR) and its modifications, that make it possible to reveal the presence of large-scale correlations

of the symbols and their groups in DNA-associated (A, C, T, G) sequences. In addition, a comparative analysis of the patterns synthesized in RY, MK, and WS bases provides a lot of additional information about the biochemical features of the sequences. However, such analysis requires further development of the methods for the quantitative analysis of point patterns with a fractal structure in terms of identifying the features of initial sequences that are important for bioinformatics. Another disadvantage of the CGR technique in its classical form is the need to use sufficiently long character sequences (from 10000 bases and over) in order to obtain sufficiently representative patterns. The FCGR modification is free of this disadvantage.

The sequence logo technique is good for identifying and visualizing the most conserved bases in the sequences during mutational changes; however, for its implementation, it requires a fairly large volume of collections of the aligned sequences. Numerous attempts at polyline representation of nucleotide sequences in 2D and 3D spaces (H-curve, C-curve, RY-curve, etc.), varying in the methods of choosing the base points in the Cartesian coordinate system and recursive algorithms for generating polyline node coordinates, have various particular advantages though they are by no means universal.

The coherent-optical methods of representing the DNA-associated symbol sequences considered here (the GB speckle technique and polarization mapping) can be classified as hybrid methods in which computer processing of the information is supplemented by integral transformations of optical information in the coherent-optical processor.

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Disclosures

The authors declare no conflict of interest.

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Modern Optical Microscopy. Part 1

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Abstract. The review presents to the reader the physical principles of various techniques of modern optical microscopy providing better quality and contrast of the image of the object under study and even overcoming the limitations caused by diffraction. These methods have proven to be a suitable tool for biomedical research. The first part of the review is devoted to the discussion of the diffraction limit of resolution, and to the description of laser confocal scanning microscopy, near-field optical microscopy, structured illumination microscopy, light-sheet microscopy, and digital holographic microscopy. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: optical microscopy; laser scanning confocal microscopy; photon tunneling microscopy; near-field scanning optical microscopy; structured illumination microscopy; light-sheet microscopy; digital holographic microscopy.

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1 Introduction

Since the end of the XVII century, optical microscopy has been an irreplaceable tool for biologists. In this review the authors consider known to date optical microscopy techniques providing better quality and informative value of the object images. The first part of the review is a discussion of the problem of resolution of a classical optical microscope and its constraints. Next, the method of confocal microscopy is considered offering the opportunity to get a better contrast and image quality for the studied samples with diffraction resolution. And fundamentals of near-field optical microscopy are also described. Then the approaches are examined where special lighting of the test sample is used. In the final fragment of part 1 of the review, the method of digital holographic microscopy is outlined, that is extremely useful for the cells study. This method makes it possible to obtain three-dimensional information about a transparent object, i.e., phase object. In the second part of the review, attention is paid to the techniques of optical microsphere nanoscopy, super-resolution fluorescence microscopy and hybrid systems combining various approaches in their structure in order to get the most complete information about the test sample.

2 Diffraction Limit of Resolution

Optical microscopy dates back to the end of the XVI and the beginning of the XVII centuries, exactly when the

first microscopes appeared. They quickly acquire the look familiar to us: there are two main optical systems in their design, the eyepiece (ocular) and the objective. In 1674, Anthony van Leeuwenhoek created a microscope with magnification, which makes it possible to distinguish and study unicellular organisms. Since then, the optical microscope has become and remains an indispensable tool for biological and medical research, due to the fact that light allows us to examine living cells without any damage.

The researchers needed to observe smaller and smaller details, and therefore the magnification of the designed microscopes increased. As magnification increases, the quality of the image obtained with a microscope stops improving after a certain value. The image of the object becomes blurry and fuzzy. The concept of optical system resolution is introduced as the ability of the system to separately represent closely located objects. The first quantitative limitation caused by the diffraction of light waves was given by Ernst Abbe in 1873 [1, 2], who worked for the company of Carl Zeiss producing optical devices. He determined the value of the minimum distance (Δx or Δy), where the two points can still be resolved:

$$\Delta x = \Delta y = \frac{\lambda}{2NA} = \frac{\lambda}{2n \sin \alpha}, \quad (1)$$

where λ is the wavelength of light, NA is the numerical aperture, n is the refractive index of the material between

the object and the objective, α is half the angle of view of the objective. In addition, there is an axial resolution limit (or the depth of field (Δz)), which is determined by the same values:

$$\Delta z = 2 \frac{\lambda}{NA^2}. \quad (2)$$

Thus, it is basically impossible to focus the electromagnetic radiation into a spot smaller than half the wavelength of light (even for the best modern immersion lenses for visible light, the transverse resolution is $\Delta x = \Delta y = 0.2\text{--}0.25\ \mu\text{m}$ and the depth of field is $\Delta z = 0.6\ \mu\text{m}$). A decrease in the radiation wavelength leads to photodamage to biological samples by ultraviolet radiation [3, 4] and requires the use of more expensive optical components [5]. The resolution problem can be solved by using electromagnetic waves with wavelengths less than the wavelengths of the optical range (electronic and X-ray microscopy). But we are not going to consider these methods here, since the aim of the review is to discuss optical microscopy techniques.

Since the end of the XIX century, a search has been carried out for methods of obtaining images with better sharpness and clarity, as well as for the ways to overcome the diffraction limit. Here it is appropriate to recall Ernst Abbe's statement regarding the expansion of the resource of the microscope as a tool for scientific research: "Modern science recognizes that the potential of our organ of vision is limited by the nature of light itself, and this limit cannot be overcome even with the help of the whole arsenal of modern natural science... The human mind will probably be able to subdue the processes and forces which will allow us to overcome in completely different ways the obstacles that now seem unconquerable. I share the hope that this will happen someday. However, I believe that the devices that will help us more than modern microscopes in our cognition of the elements of the material world, will have nothing to do with these modern microscopes, except for their name" [1, 6]. As the matter of fact, the capabilities of modern optical microscopes would not be so impressive without the development of computer technologies and new types of devices for recording images and their subsequent computer-aided processing, that makes modern microscopes hybrid systems [5, 7]. The techniques of optical microscopy capable of raising the quality and resolution of images and overcoming the diffraction limit are discussed in detail below.

3 Confocal Microscopy

Our consideration of ways to improve the quality of the image formed by an optical microscope begins with the method of confocal microscopy. Marvin Minsky, the author of the first patent (1957) for a confocal microscope [8], proposed the method. The method of optical confocal microscopy provides a more contrasting image of the test sample in comparison with a classical microscope. This method offers the most successful

results in case of relatively thick samples under study [9]. The thickness of the sample is tens of micrometers. These are sections of brain tissue, embryos, whole organs and small organisms. An increase in the image contrast is achieved by using a small aperture that filters the light, its diameter ranges from tens to hundreds of micrometers. As a result, only a small volume near the focus point of the lens is displayed, since the aperture cuts off the illumination of the sample layers located above and below. This method is clearly seen in Fig. 1, which shows the trajectory of the rays in the classical and confocal scheme of microscope. In order to obtain a high-contrast image of the whole sample, it is necessary to scan it while re-configuring the system or moving the sample.

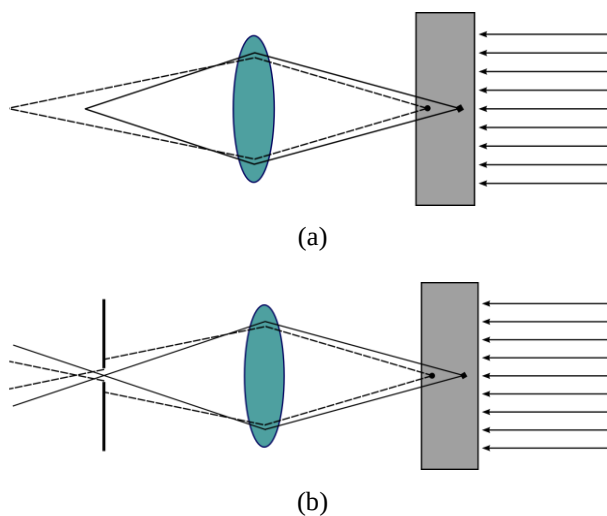


Fig. 1 Sample illumination: (a) ray path in the classical scheme of the microscope, (b) ray path in a confocal microscope.

Another distinctive feature of the confocal microscope is that it illuminates only the volume to be studied [9]. This can be achieved by equipping the confocal microscope scheme with a second focusing system placed behind the sample (Fig. 2(a)). But this scheme requires an additional restriction on the test samples – they must be transparent. Objectives are rather expensive optical elements, and the use of a second focusing system for the sample illumination raises the cost of the entire system. To avoid this, an alternative scheme for the sample illumination was found where a beam-splitting plate was used. In this scheme, a single objective is used to illuminate and look the sample (Fig. 2(b)). This configuration was proposed by M. Minsky [8]. It is more accessible than the previous one and besides it facilitates the adjustment of the confocal system.

The beginning of serial production of confocal microscopes dates back to the 80s of the XX century, being associated with the development of laser and computing technologies. Replacing the lamps, the laser becomes the source of the radiation that excites fluorescence in the sample. To obtain three-dimensional information on the sample, its scanning is carried out.

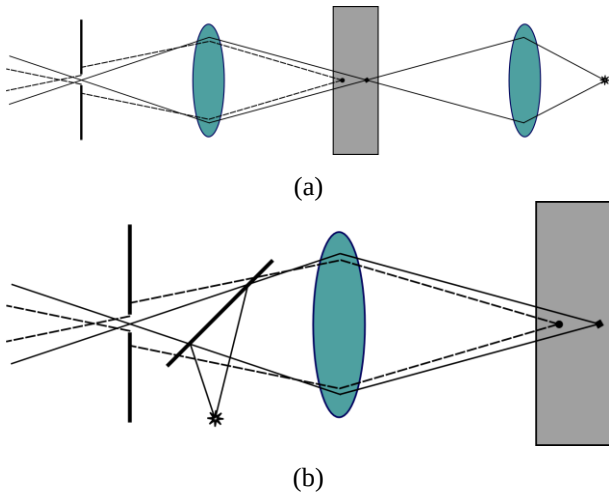


Fig. 2 The sample illumination in confocal microscopy: (a) with use two focusing systems, (b) with use of one focusing system.

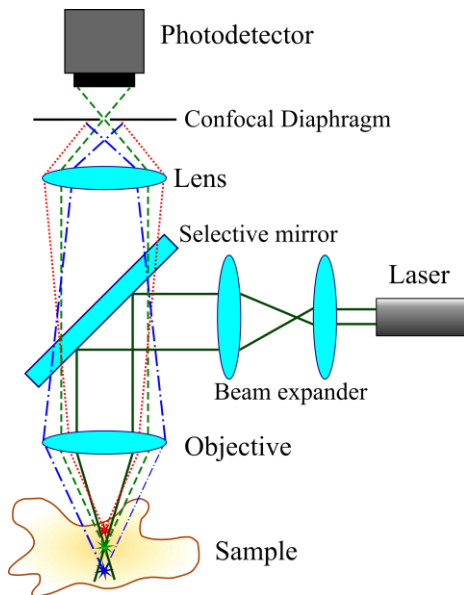


Fig. 3 Principle of the confocal signal filtering.

These features are reflected in the name of the method – Laser Scanning Confocal Microscopy (LSCM) [10–13]. Let us consider a fundamental scheme of a modern LSCM-system. To illuminate the sample, a scheme is developed that uses an objective both to obtain an image and simultaneously to illuminate the sample (Fig. 2(b)). Laser radiation is directed onto the sample through the objective using a dielectric selective mirror (Fig. 3). The use of a selective mirror allows spectral filtering of radiation, the light from the laser will not penetrate into the channel where the sample fluorescence is registered. The laser radiation is focused by the objective and is exciting fluorescence within the volume of the test sample under study (solid lines in Fig. 3). Induced fluorescence of the sample (dotted lines in Fig. 3) is collected by the objective, the same one used for the sample illumination. The fluorescent radiation excited in the layers located above and below the focal plane is cut

off by confocal diaphragm. Only the part of the sample fluorescence that is emitted from a small volume of the sample close to the focus area of the laser beam under the objective-passes through the diaphragm (Fig. 3) [12].

The diameter of the confocal diaphragm D determines the thickness of the optical layer of the sample Δz near the focus of the objective, within the boundaries of which the signal is measured. The thickness of the optical layer Δz near the focus is defined by the expression [12]:

$$\Delta z = \sqrt{\left(\frac{0.88\lambda_{em}}{n - \sqrt{n^2 - NA^2}} \right)^2 + 2n^2 \frac{(D/\Gamma)^2}{NA^2}}, \quad (3)$$

where λ_{em} is the wavelength of fluorescence emission, n is refractive index of the medium between the objective and the sample under study, D is confocal aperture diameter, NA is numerical aperture of the objective, Γ is magnification of the optical system of the microscope between the objective focal plane and the conjugate focal plane where the confocal diaphragm is located. By changing the size of the confocal diaphragm aimed to increase the longitudinal resolution, a situation can be achieved in which a decrease in the diaphragm diameter no longer causes an increase of resolution in the longitudinal direction. The optimal ratio between the light power and resolution is achieved in case of equality of the confocal diaphragm diameter to the first diffraction maximum, while the best resolution is obtained when the diameter of the confocal diaphragm is equal to 5–10 times the size of the Airy disk. Diaphragms of this size are used in modern confocal microscopes [11, 12]. The confocal diaphragm on the fluorescence path from the sample to the detector changes the point spread function (PSF) of the optical system. The PSF becomes narrower.

The resolution in the transverse direction (XOY plane) for a confocal microscope is determined by the FWHM parameter (Full Width at Half Maximum of the point spread function) [11, 12]:

$$\Delta x = \Delta y = \frac{0.51\lambda_{em}}{NA}. \quad (4)$$

The following values of the parameters included in Δx and Δz , are typical for systems with the immersion lens: $n = 1.5$, $NA = 1.3$, the wavelength of the sample fluorescence $\lambda_{em} = 540$ nm. The estimation of the transverse and longitudinal resolution gives the following values: $\Delta x = 0.21 \mu m$ and $\Delta z = 1.06 \mu m$. That is, they have the order of values obtained by Eqs. (1) and (2), and do not exceed the diffraction resolution limit.

A successive scanning of the sample by re-adjusting of the optical system and/or by moving the sample allows you to restore the three-dimensional distribution of fluorescent labels over the sample. The sensitivity of modern microscopes and the scanning speed are high enough to study the dynamic and kinetic transformations occurring in living samples. Under intensive

fluorescence, modern scanning microscopes are able to measure an image of 512×512 pixels size in 200 ms. Lower resolution images are registered faster. For experiments with alive samples, special incubators have been developed that are mounted directly into the microscope system and permit to conduct hours-long research at specified temperature, concentration of substances and elements needed for the sample [10].

LSCM is a method of multidimensional microscopy since a three-dimensional XYZ data array is formed during the registration of the spatial distribution of fluorophores in the sample. When repeating XYZ-data measurements, a four-dimensional XYZt-data array is obtained. It is possible to add a fifth dimension if a spectral approach is used to analyze the received fluorescence signals [10].

The fluorescence of the sample in combination with the confocal system allowed for three-dimensional visualization of living cells and tissue slices [6]. This combination has turned the optical microscope into one of the most powerful and multifunctional tools for solving the problems in modern cell biology, for example, for studying bacterial biofilms [13, 14]. But even the use of high-quality optics does not eliminate the limitation imposed on the resolution of a fluorescent confocal microscope by the wave nature of light.

Confocal microscopy methods and equipment continue to evolve. To increase the field of view with homogeneous illumination it was proposed in Ref. [15] to use an array of micro-lenses with a spatial light modulator. A prototype of the system was built with the field of view of $3.5 \text{ mm} \times 3.5 \text{ mm}$ -size, illuminated by 2500 confocal points. The system provides a lateral resolution of $\sim 0.82 \mu\text{m}$, and the resolution increase is about 1.6 times. The unevenness across the total image area is 3%.

To increase the resolution in the axial direction, 4Pi scanning confocal microscopy was proposed [16–18]. The basic idea of the confocal fluorescence 4Pi-microscope is to use two microscope lenses with a common focus providing the increase in the microscope aperture [16–20] (Fig. 4). The radiation from the exciting laser source is divided in two beams and directed to micro-objectives. A standing wave is formed in the focus area. By changing the length of the optical path in one of the arms of the interferometer, it is possible to achieve the coincidence of the phases of the two waves, i.e., constructive interference. This results in a narrowing of the point spread function in the longitudinal direction and, accordingly, in an increase of resolution.

In the first version of the 4Pi fluorescence microscope system, the obtained resolution value was 110 nm according to the FWHM criterion, which is 4 times lower than the resolution of the confocal fluorescence microscope. Thus, it becomes possible to resolve smaller details if compared with the confocal fluorescence microscope. It was also shown that the 4Pi-microscope is capable of operating in two-photon excitation mode, thus further improving the achievable resolution [19]. The overall advantage of the 4Pi microscope is that, due to the

formation of interference, only half the light intensity is required to expose the sample, so it provides better protection against unfocused bleaching of fluorophores. Besides, it can achieve the same intensity and signal-to-noise ratio as a confocal fluorescence microscope system, but with a smaller volume of the fluorophore under study. Since the solid angle of 4π is not practically attainable, the term 4Pi was chosen to denote the basic idea of the method. In addition to working as a confocal fluorescence microscope, the confocal fluorescence 4Pi-microscope can form three different types of images with a higher resolution. The first type is the interference of two illumination waves; the second type is the interference of two recorded waves on the detector; and the third type is interference of illuminating waves and recorded ones. There was also available a similar I⁵M-microscopy technique [20, 21]. Due to the sophisticated technology, subsequent mathematical processing of images, and inconvenience of working with biological samples placed between close-standing objectives, these microscopic systems are not widely used.

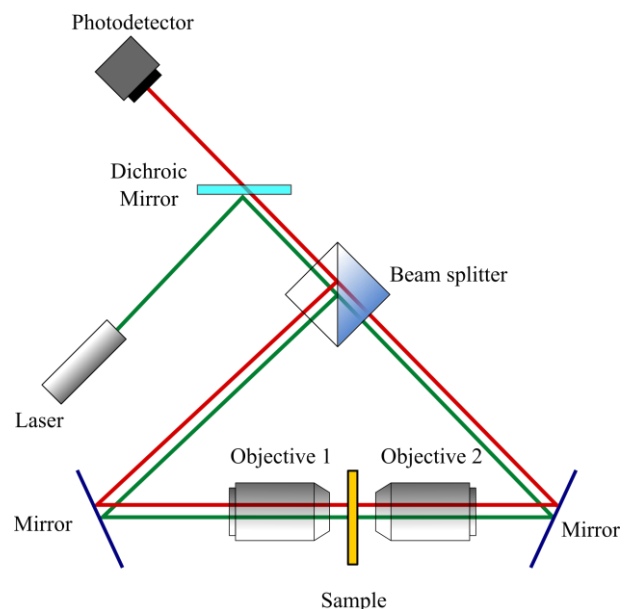


Fig. 4 Optical layout of a confocal 4Pi-microscope.

4 Near-Field Scanning Optical Microscopy

In this chapter the techniques of near-field optical microscopy providing the possibility of obtaining super-resolution with no fluorophores are reviewed. Near-field optical microscopy is one of the options of scanning probe microscopy [22–25]. And in turn, scanning probe microscopy (SPM) is one of the powerful modern techniques for studying the morphology and local properties of the solid surface with high spatial resolution [22]. Currently, it is a widespread and effectively used tool for studying the surface properties.

As already mentioned, the resolution of a classical optical microscope is restricted by the diffraction limit, (Eqs. (1) and (2)). In near-field optical microscopy, other principles of the object imaging are used that make it

possible to avoid the difficulties caused by the light diffraction and achieve the spatial resolution level as high as 10 nm and still better.

The work of near-field microscopes is based on the light passage through subwavelength diaphragms. The electromagnetic field within the area of the subwavelength diaphragm has a complex structure. Directly behind the aperture at distances $Z < 100 \text{ \AA}$ the near zone is located, where the electromagnetic field exists mainly in the form of evanescent waves localized close to the diaphragm surface. The evanescent waves are non-propagating electromagnetic waves with their energy concentrated near the source. Therefore, they exponentially decay while moving away from the Ref. [26] (Fig. 5). Owing to this behavior they can be only recorded by placing near the sample the probe that will act as a converter of evanescent waves into-emitting modes whose intensity can be measured by a photodetector. Being tiny, the probe limits the field of view, therefore, in order to examine a large sample, it should be scanned.

A near-field optical microscope was invented by D. W. Pohl, working at the laboratories of IBM in Switzerland, in 1982, following the invention of the tunneling microscope [26]. The small radius aperture, $r = 30 \text{ nm}$, was made on the tip of a quartz crystal rod of 100 mm-length and 2 mm-thickness. The resolution of the first near-field optical microscope using laser radiation at a wavelength of $\lambda = 488 \text{ nm}$ was 5–10 nm. The further development of the method led to the emergence of new types of transducers and techniques for scanning the sample [25–31]. There are several ways of the sample scanning (Fig. 6). In the initial version, a subwavelength aperture is used, the sample is placed on a hemispherical substrate (Fig. 6(a)), and a part of its surface is illuminated [25, 28]. The glass hemisphere acts as a converter and transmits the signal collected in the near field to the detector in the far field. The transverse resolution obtained for this configuration ranged from 10 to 100 nm. Other versions of the scheme configuration have also been proposed (Fig. 6(b)), they are aperture-free or Tip-Enhanced Near-field Optical Microscopy (TENOM) [26, 29–31]. The external illuminating field is

captured by the probe end and amplified, thus making it possible to convert evanescent waves in the sample into emitting modes with higher efficiency. The cross-sectional resolution is up to $1\div 20 \text{ nm}$, but in this case the useful signal is superimposed on the background of the illuminating beam. The third variant of this method consists in scanning with a dielectric tip without covering the near field in the immediate vicinity from the sample (Fig. 6(c)). This method is called Scanning Tunneling Optical Microscopy (STOM) [26, 31] or Photon Scanning Tunneling Microscopy (PSTM).

The photon tunneling microscopy is also referred to methods of near-field optical microscopy. The resolution of this method in the XOY plane is $\sim 0,29\lambda$ [26], which is almost half the diffraction limit.

Methods of near-field optical microscopy [26] are widely used to obtain thin-films images, diffraction gratings, and also in near-field Raman spectroscopy, in pulsed laser ablation through probes, etc. However, these techniques are characterized by a very small working distance (the distance between the probe and the test surface does not exceed $\lambda/2$, approximately 1–10 nm), an extremely shallow depth of field (150 nm) and a long scanning time for large areas of the sample, so, for example a $1 \mu\text{m}^2$ area is scanned in 16.7 min. These features limit the scope of the method application.

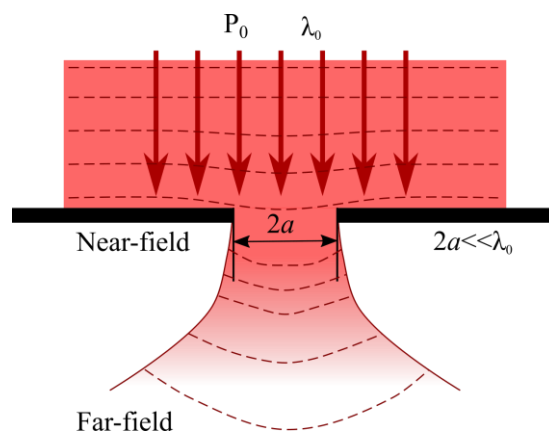


Fig. 5 Evanescent waves.

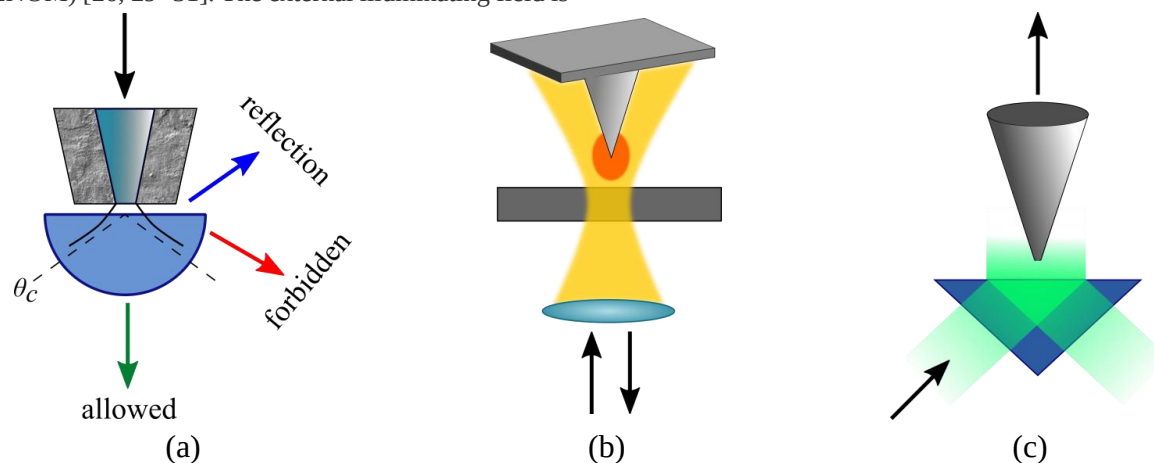


Fig. 6 Options of practical implementation for near-field scanning microscopy: a) based on a subwavelength aperture, b) tip-enhanced near-field optical microscopy, and c) photon scanning microscopy.

5 Microscopy with Special Illumination of the Sample

In order to increase the transverse magnification of microscopes, it was proposed to use special illumination of the studied samples. It is possible to achieve a transverse resolution greater than the classical Abbe's limit without reducing the field of view and without getting rid of background radiation, but instead using lateral illumination with a special structure of intensity distribution in a microscope with a wide field of view. The structure of the intensity distribution of the illuminating beam is set in a special way. In a method called Structured Illumination Microscopy (SIM), the sample is illuminated by spatially structured exciting light, which makes normally inaccessible high-resolution information visible on the image record in the form of moire fringes. The spatial structure of the beam illuminating the sample is formed by a diffraction grating and is a sinusoidal distribution with the period close to the resolution limit of the micro-objective used. A series of such images is then processed and as a result, the image of an object with an improved resolution is obtained [32–34].

To evaluate the functionality of the method, the Fourier transform should be considered. In the spatial spectrum, low-resolution information (low frequencies) is close to the center (zero frequency) while higher-resolution information (high frequencies) is further away. A conventional microscope can resolve structures of samples no smaller than the diffraction limit $\Delta x = 0.2 \mu\text{m}$. Therefore, the microscope can detect only the information located in the spectral zone enclosed by a circle of $1/\Delta x$ -radius centered at the origin of coordinates. This will be the observed region (Fig. 7). Red circle is the diffraction limit of $k_{em} = 2NA/\lambda_{em}$, λ_{em} is the wavelength of the fluorescence emission and NA is the numerical aperture of the microscope objective. The structured illumination of the sample will not change the size of this physically monitored area, but it will facilitate the movement of information from the high frequency region (smaller details) and make this information observable. As a concrete example, the structured exciting illumination in the form of a strip with a sinusoidal profile of the intensity distribution, obtained with a diffraction grating is considered (Fig. 7(a), setup diagram). Its Fourier transform will have three non-zero points (Fig. 7(b), spatial frequency $k_{ex} = 2NA/\lambda_{ex}$, λ_{ex} is the wavelength of the exciting illumination). One of these points is located at the origin of coordinates and the other two are shifted relative to the origin in the direction determined by the orientation of the stripe and by the distance inversely proportional to the width of the stripe. Under the sample lighting with structured illumination, the resulting image of the object will also contain high spatial frequencies (Fig. 7, reading in the limited frequency range, $k-k_{ex}$ and $k+k_{ex}$). The parts of these shifted circles that go beyond the normally observed region, provide new information that is not available via an ordinary classical microscope.

The observed image is the sum of three components, and they cannot be separated by using a single image. However, the coefficients by which they are summed up, depend on the known phase of the structured illumination which can be controllable. By recording three or more images of a sample with different lighting phases, the three components can be separated by calculations, and information on the sample structure can be restored (Fig. 7(b)).

Provided that the extremely large shift distance is selected and the lighting scheme is configured as accurately as possible, then it becomes possible to double the image resolution in the direction of structured illumination. By repeating this procedure with multidirectional lighting, it is possible to collect almost all the information within a circle twice as large as the physically observed area (Fig. 7(b)). The complete information obtained in this way will help to restore the image of the sample with a resolution twice as high.

Comparison of the resolution of structured illumination microscopy with other approaches shows a twofold gain [32]. When examining the sample, which is a cluster of fluorescent microspheres of 121 nm-diameter, the size of microspheres measured using three types of microscopies, i.e., classical microscope, confocal microscope and structured illumination microscopy was 290 nm, 210 nm, and 130 nm respectively. So, the last size measured using the structured illumination microscopy is closest to the sample parameters. Thus, the results confirmed the expected increase in resolution when using this method compared to conventional microscopy and confocal microscopy. And the confocal microscope worked in the maximum resolution mode.

The method of structured illumination microscopy is compatible with existing modern optical microscopes, which makes the technique accessible. This method has been further developed over the past two decades in the form of various approaches with their advantages and shortcomings; it has become a key illumination-method for optical slices, for ultra-high-resolution imaging, surface profiling and quantitative phase visualization of micro-objects in cell biology and engineering. The work [34] can be considered a practical guide for biologists; it attempts to answer the question: which approach is best suited for these studies.

Another approach of using special sample illumination is Light-Sheet Microscopy (LSM). The idea of such a visualization method appeared and has been brought to realization over the past 20 years. The name of the method reflects the basic concept of illuminating the sample planes with sheets or layers of light and using subsequent scanning of the sample to construct a three-dimensional image. This approach has a number of obvious advantages, such as a significant reduction in the dose of optical radiation per sample, and simplicity of large-format visualization at high speed [35]. The method has the capability to meet the criteria required to obtain a large-scale three-dimensional image with a perfect space-time resolution.

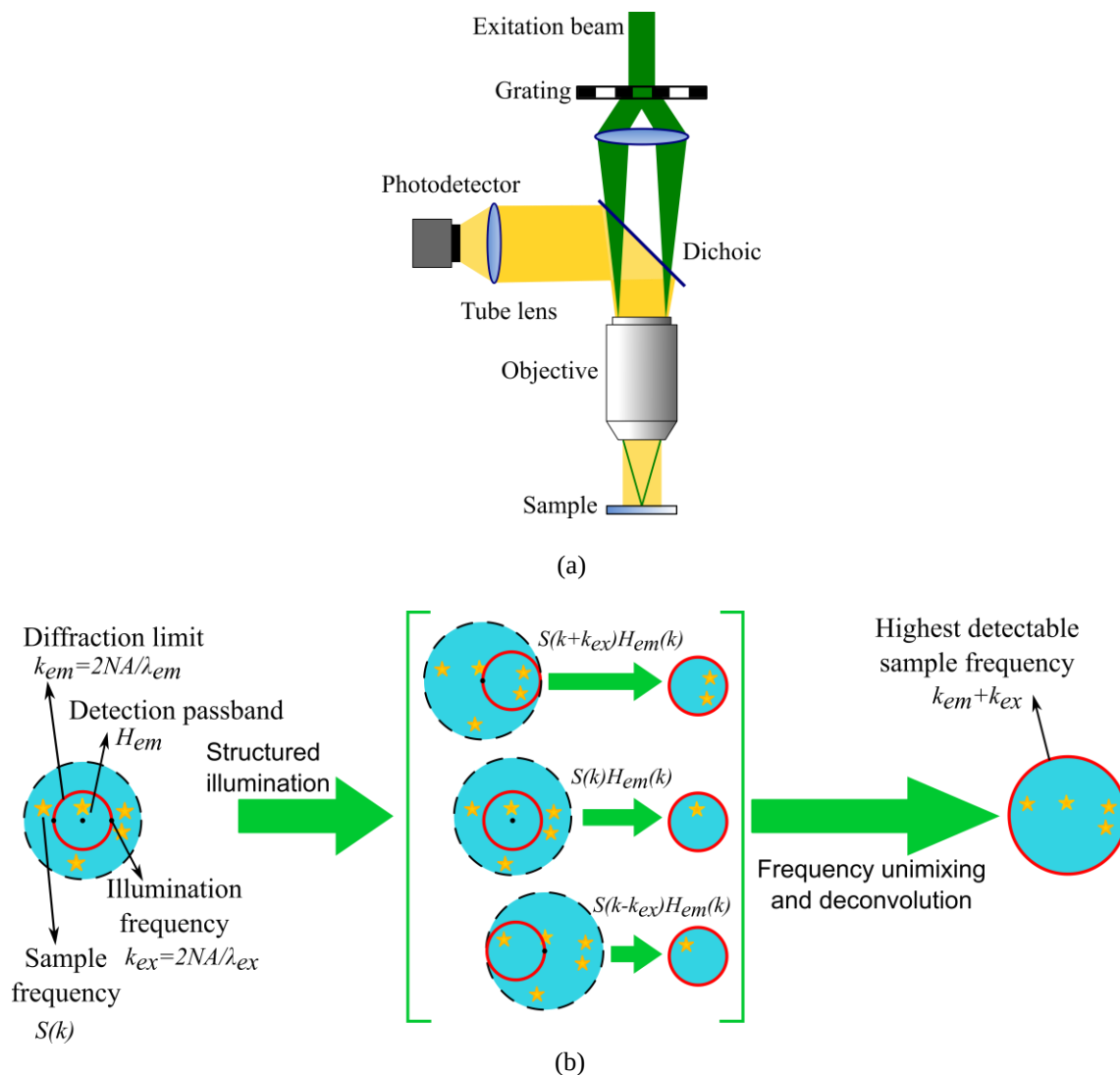


Fig. 7 The concept of the resolution increase as a result of the structured illumination. (a) Schematic diagram of Structured Illumination Microscopy. (b) The red circle in the figure on the left limits the resolvable spatial spectrum of the fluorescent emission of the sample. Maximum spatial frequency determined by diffraction limit is $k_{em} = 2NA/\lambda_{em}$. The diffraction grating creates an interference pattern in the plane of the sample with a spatial frequency k_{ex} . This leads to a shift in the spatial frequency of the sample spectrum $S(k)$ in $S(k+k_{ex})$ or $S(k-k_{ex})$. It permits to register higher frequencies in the detection bandwidth $H_{em}(k)$ of the system. After computational processing, the maximum frequency of the resulting image can be increased to $k_{em} + k_{ex}$.

In the most fundamental sense, the LSM-method is based on selective illumination of the sample [36, 37]. The fluorescence of labels lying outside the focal plane reduces the contrast of the formed image. For this reason, the sample is illuminated only within the focal plane. The sample is illuminated by a separate optical system placed perpendicular to the optics collecting the fluorescence radiation of the sample. A beam weakly focused by a lens, illuminates volume of an approximately cylindrical form with dimensions that remain relatively unchanged in the cross-section in the focal plane at a distance equal to the Rayleigh length of the illuminating beam [38]. To get an image from the entire focal plane at one go, the beam should be expanded along one axis. In the first systems of this type, it was achieved by means of a cylindrical lens that focuses the beam in only one

transverse direction. Such a system called microscopy of selective plane illumination, its schematic diagram shown in Fig. 8(A) [36, 37]. The light sheet in such a system has a Gaussian envelope along its entire length. A light quasi-sheet can be obtained by the sample fast scanning with a beam focused by a spherical lens. Such a system is called digitally scanned light-sheet microscopy [39, 40]. In case when the sample scanning by a beam takes less time than the integration time (i.e., exposure time) of the camera used to obtain the image of the label's fluorescence, then the scanning beam will be perceived by the camera as a continuous sheet [38]. In this configuration, the light sheet has a more homogeneous intensity profile all along its length, thus reducing the noise generated in the case of coherent light [40].

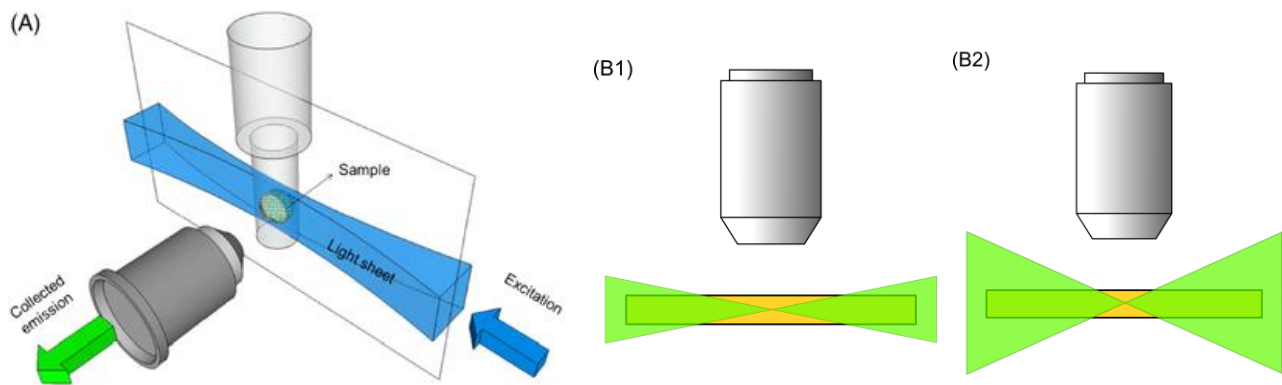


Fig. 8 Light-Sheet Microscopy. Schematic diagram. (A) Microscopy of selective plane illumination. Reprinted from Ref. [41], Copyright (2020), with permission from John Wiley and Sons. Sample lighting options: x-z views of low (B1) and high (B2) NA.

The resolution of a microscope with the light sheet's illumination will be determined by the Abbe criterion (1) and (2). However, there are two modes of operation that determine the axial resolution of the system. The first one consists in generation of a weakly focused and relatively thick light sheet illuminating the sample. The thickness of such a light sheet is approximately equal to the axial resolution of the micro-objective forming the image of the sample. Under such illumination the axial resolution will coincide with that of a standard microscope, but the generated image will have a high-quality contrast, since the fluorescent labels above and below the focal plane are not emitting light (Fig. 8(B1)) [35–37]. While increasing the thickness of the light sheet, you can save the resolution, but the contrast of the image will gradually decrease. Another running regime consists in making the thickness of the light sheet as narrow as possible compared with the axial resolution of the lens [42–44]. In this case, the axial resolution is determined by the light sheet thickness, Fig. 8(B2). With this configuration, a uniform high spatial resolution is achievable, but a sharper focusing of the light sheet is required. This configuration allows for uniform high spatial resolution, but requires sharper focusing of the light sheet. With sharp focusing, the natural divergence of the light sheet narrows the field of view where a homogeneous resolution can be achieved. This will also result in a change of the spatial resolution of the generated image across the field of view of the system.

On the contrary, by using a wide light sheet with low divergence (i.e., retaining its size), it is possible to get an image with the same spatial resolution and contrast all over the field of view. The light sheets microscopy is suitable for volumetric imaging, because it permits to obtain an optical cross-section of the sample by separating signals from z-shifted planes in the sample. The optical cross section can be also obtained by confocal microscopy and structured illumination methods described above. But for these techniques scanning of the sample (in case of confocal microscopy) or obtaining multiple images (in structured illumination microscopy) is required, which makes them slower

compared to the light sheet microscopy providing an optical cross-section of the sample in one measurement. In microscopy of light sheets, special beams are used that are invariant to propagation, for example Bessel, Airy beams owing to specific properties of structure of these fields. Such fields are also called non-diffractive. The Bessel beam amplitude is described by the Bessel function of the first kind, and its central peak of intensity does not expand during propagation, unlike a Gaussian beam. And Airy beams have similar properties. Their use makes it possible to obtain a narrow light sheet within a larger area, therefore a high spatial resolution is maintained in a large field of view. Besides, their use makes it also possible to improve the quality of the generated image while working with thick highly scattering samples [35].

The main drawback of this method remains its dissemination to a relatively small audience of end users. Most modern systems of light sheet microscopy require spatial light modulators inside the system, which complicate and raise the cost of the system design [45].

The problem can be solved by replacement of the controllable modulators with stationary elements. The development of methods for the aberration correction is also carried out. New promising opportunities for application of this method in studies of dim biological samples are opened up owing to multiphoton excitation of fluorescent labels.

There is still a great number of issues in microscopy of light sheets that need to be solved and worked out, but they are not mentioned in this review.

They are related to technical problems and constraints of the method. The most significant of these issues is the use of multiview systems, including polygonal and two-way illumination and/or fluorescence detection [46–49], which combine several data sets to obtain a high-quality image of the whole sample; oblique-plane illumination systems [50], capable of creating a light sheet and collecting fluorescence through a single lens. Although these system configurations provide important advantages, they still use the Gaussian beam and face the restrictions that it imposes.

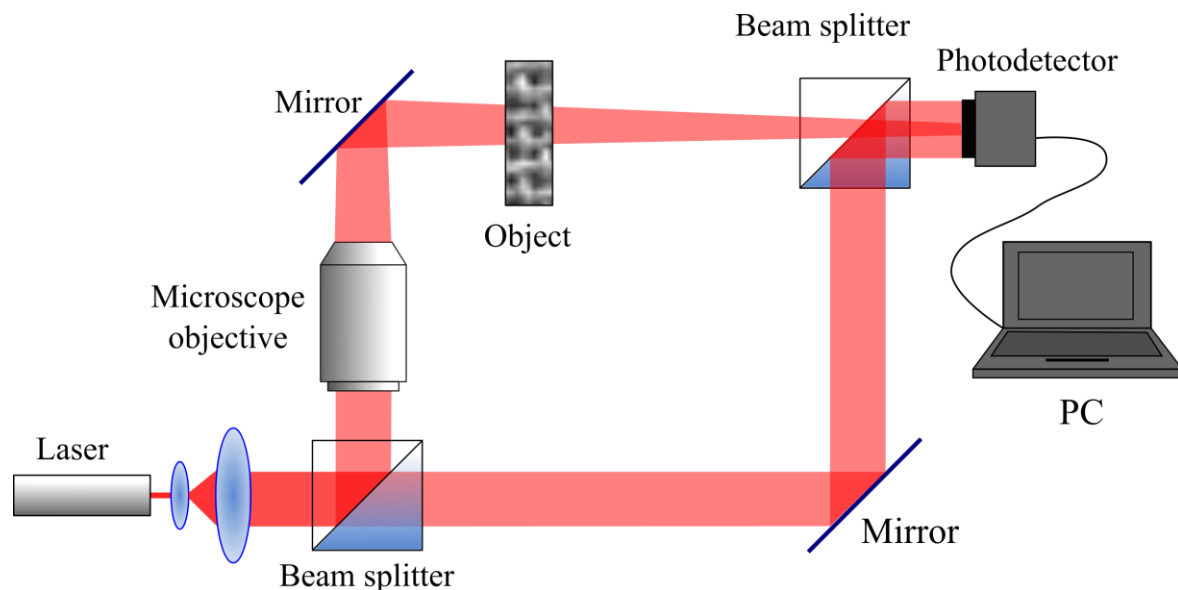


Fig. 9 Schematic diagram of the setup for recording holograms in holographic microscopy.

All these systems will acquire new advantages due to structured light approaches and use of Bessel or Airy beams in the near future. The article [51] discusses diverse applications of fluorescence microscopy of light sheets (Light sheet fluorescence microscopy (LSFM)) for visualization of individual cells and various multicellular samples, as well as a combination of LSFM with other imaging approaches that provide a larger working depth and ultra-high resolution, or the use of complex space-time manipulations providing observations in several directions.

6 Digital Holographic Microscopy

Recently, Digital Holographic Microscopy (DHM) is also successfully developed [52–55]. This method allows you to get a three-dimensional image of the sample being studied. Most of the components of a living cell are almost entirely transparent for the visible wavelength range, so the image obtained with a classical microscope has a poor contrast. However, the refractive index of different parts of the sample cell differs, and therefore the information about the sample will be contained in the change of the phase reflected from the sample or from the light wave that has passed through the sample. The hologram recording in digital holography is carried out using a photodetector device (digital camera), during registration of the resulting field, while this field is an interference of the reference wave and object waves. The hologram contains comprehensive information about the three-dimensional distribution of the optical field of the object wave in the form of the interference fringes. The process of recovery of the original object image in classical holography consists in illumination of the resulting interferogram (i.e. hologram) with a reference wave, and due to diffraction, the image of the object is restored. As for digital holography, here to reconstruct the object image, numerical methods are used, which make it possible to evaluate the amplitude and phase of

the object wave by means of the hologram processing. The main feature of digital holographic microscopy is the use of an additional optical system, i.e., micro-objective between the object and the photodetector (PD) so that to form an enlarged image of the interference area (Fig. 9). This is the main difference between the digital holographic microscopy and just digital microscopy. For the transparent samples study, the hologram is registered in a scheme with a Mach-Zehnder interferometer, whereas for the study of reflective samples – with Michelson interferometer (Fig. 9). Both lasers and sources with a low coherence, for example, super luminescent diodes, can serve as radiation sources.

For the method of digital holographic microscopy does not require precise focusing, which makes the system more accessible due to the reduction in the number of expensive optical and mechanical components in it. The hologram is recorded at a fixed position of the lens, and focusing at different distances is fulfilled using special computational algorithms. The image making is based on a new technique of computer data processing. The signal from the interferometer is processed in real time, and three-dimensional image of the object is concurrently built up.

With the development of inexpensive fast CMOS (Complementary metal–oxide–semiconductor) matrices, powerful algorithms for image reconstruction and phase search, in combination with lowering the cost of computation, the concept of lens-free holographic microscopy has become an attractive alternative to traditional digital holographic microscopy. Over the past 10 years, due to reduction in the cost and complexity of devices without any damage to resolution and image quality, the lens-free technology became more relevant for practical use, and adaptable for applied tasks. In Ref. [52], a lens-free holographic microscope is described, capable of distinguishing objects with the thickness ranging from 350 to 50 nm and with a signal-to-noise phase ratio of 23 dB. High resolution is

achievable due to the improvement of numerical algorithms for reconstructing a three-dimensional image of the object under study by a recorded digital hologram. Studies are underway to develop various ways to improve the spatial resolution of such systems.

7 Conclusion

The first part of the review is devoted to the consideration of optical microscopy techniques, widely used in biological and medical research as well-proven and reliable tools. Confocal microscopy opens an opportunity to obtain images with high contrast, and three-dimensional scanning makes it possible to restore the volumetric structure of the studied object. With the aid of near-field optical microscopy, images of surfaces and objects can be observed with a resolution below the diffraction limit via detecting of attenuated waves. Structured illumination microscopy provides an increase

in resolution of the resulting image due to special illumination of the sample and subsequent processing of the obtained images. Light sheets microscopy opens the way to obtain information about the volumetric structure of an object. Depending on the mode, either its axial resolution can be higher than diffraction limits, or the resulting image can have a better contrast compared to classical microscopy. Digital holographic microscopy has become a new well-proven method in biological applications, owing to which it became possible to carry out the volumetric reconstruction of transparent objects and analysis of their structure.

In the second part of the review, it is proposed to discuss optical techniques that give spatial resolution above the diffraction limit.

Disclosures

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Calculation of the Interference Coefficient of the Polyatomic Molecular Structure of DNA

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Abstract. This paper shows the calculation of the DNA interference coefficient and uses it to obtain the spectrum of the interaction of an ultrashort laser pulse with a molecule. The calculation is based on a simplified model of the molecule.
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Keywords: laser pulses; molecule; DNA; radiation spectrum.

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1 Introduction

Currently, much attention is focused on the study of complex molecular structures using ultrashort laser pulses [1, 2]. This is due to the difficulties of analyzing unsteady molecular structures using other methods [3, 4]. Methods from the field of chemistry, the Slinger method, for example, give accurate results, but the study is long and time-consuming. X-ray irradiation does not give accurate results. That is why scientists are now interested in the use of ultrashort laser pulses [5, 6]. With their help in the future it is possible not only to determine the structure of multi-atomic objects, but also to study of molecular processes in it [7, 8], because ultrashort pulses operate in a very small time range, corresponding to atto and femtosecond scales.

The problem with using ultrashort lasers today is primarily the lack of a theoretical apparatus for interpreting the results obtained experimentally. Deciphering the interaction spectra is complicated without theoretical calculations of the process of irradiating a molecule with laser pulses. There are ways to calculate the interaction of pulses with one target, one atom or molecule consisting of atoms of one substance. However, there are molecular objects, interesting for research and consisting of many atoms. The calculation in this case is complicated by the number of atoms, different electron density, over-radiation, etc. Such an object can be calculated on a qualitative level, using simulation methods, specifying the coordinates of the atoms included in the system [9]. However, the calculation of the system defined by a real-DNA number of coordinates requires a large amount of time for processing (more than a day), which is inconvenient for analysis. To simplify, it is necessary to move away from the use of coordinates and introduce an analytical

formula for the spectrum of interaction of ultrashort pulses with the object. The main component of the spectrum formula is the interference factor. It changes depending on the spatial position of the elements in the system. It is impossible to describe it analytically for a real molecule, consisting of many atoms. This paper proposes a simplified model of a DNA molecule, which is used to calculate the interference factor.

2 Theory

In this work, we will use a single helix of DNA as the object of study; this will facilitate the process of considering the atomic model of the molecule. We use a DNA helix with alternating nitrogenous bases: cytosine and guanine (Fig. 1).

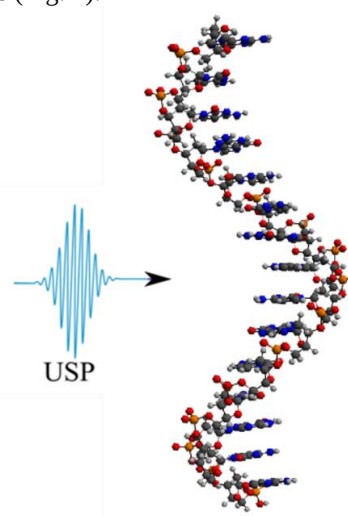


Fig. 1 The helix of a DNA molecule irradiated by an ultrashort laser pulse (USP). Performed in the Avogadro program.

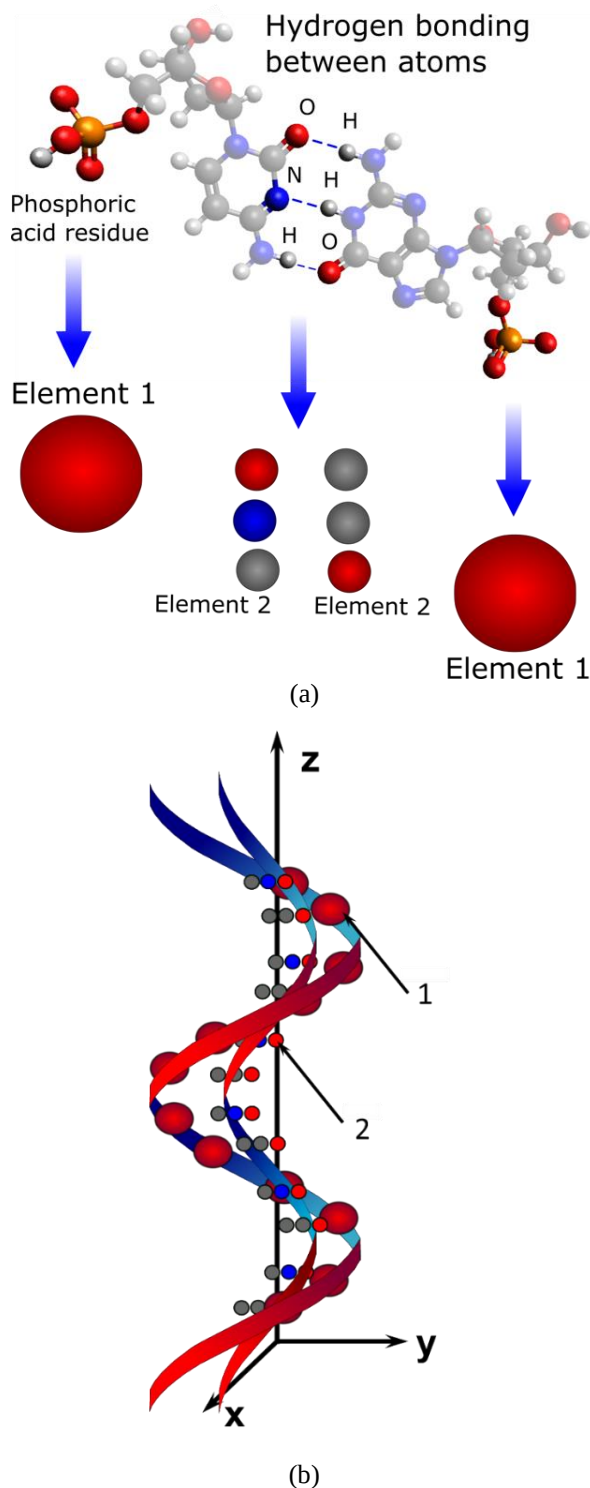


Fig. 2 A simplified model of a single turn of the DNA helix. Atoms involved in hydrogen bonding: gray – hydrogen, red – oxygen, blue – nitrogen. a) An illustration of how the elements were formed. b) One turn of the spiral built with the elements.

According to the principle of complementarity, only they can interact with each other, so the presence of adenine and thymine next to each other is excluded. Suppose an ultrashort laser pulse falls on a molecule. In this case, there are many atoms in the radiation path that

have some symmetry and periodicity. The periodicity of the structure of the molecule makes it possible to simplify the model and move from a polyatomic structure to a system consisting of simpler elements.

Each nitrogen base of the molecule is connected to the opposite one by three hydrogen bonds. The DNA helix consists of a phosphoric acid residue and a monosaccharide. Thus, these elements perform an important function, they “form” a molecule. The remaining atoms from the analysis can be omitted. Thus, the outer part of DNA turns into a periodic set of elements as shown in Fig. 2, where 1 denotes monosaccharide + phosphoric acid residue and 2 denotes the inner: nitrogenous base atoms involved in hydrogen bonding. The remaining atoms that make up the molecule are not considered, we believe that in the absence of elements 1 and 2, the remaining elements are also missing, because the “connection” function is not performed.

Based on the calculations given in Refs. [9, 10], we write down an expression for the interference factor:

$$g_N(p) = \left| \sum_a e^{i \cdot p R_a} \right|^2, \quad (1)$$

where p – scattering pulse, i.e. the pulse received by an electron when scattering an ultrashort laser pulse, a – system atoms, R_a – radius-vector of the atom. In the case of simplification, the atom is replaced by an element and we consider elements of two varieties 1 and 2.

In general terms, the radius vector is represented by the following expression:

$$R_a = xi + yj + zk, \quad (2)$$

$$\begin{cases} x = \rho \cos \varphi \\ y = \rho \sin \varphi, \\ z = z \end{cases}$$

where $\rho = R_a = \text{const}$ is a radius of the plane of rotation of a single element, $\varphi \in [0; 2\pi \times T]$ – rotation angle. The nucleotide rotates in one plane on a circle, so the angle can be written as $\varphi = 2\pi \frac{a}{N_T}$, where a – sequence number of the nucleotide, N_T – their total number for the

period. $z = C \cdot 2\pi \frac{a}{N_T}$, where C is the geometric parameter of the spiral. Then we change from a system consisting of atoms to a system consisting of elements and replace the radius vector designation R_a with R_l .

In the case of simplification, we get two spirals, external and internal. They differ only in the number of elements and radius-vectors. Let us locate both spirals in the xyz coordinate system (Fig. 3).

In Fig. 3, R_1 is the radius-vector of external elements (monosaccharides + phosphoric acid residues) and R_2 is the radius-vector of internal elements (nitrogenous bases).

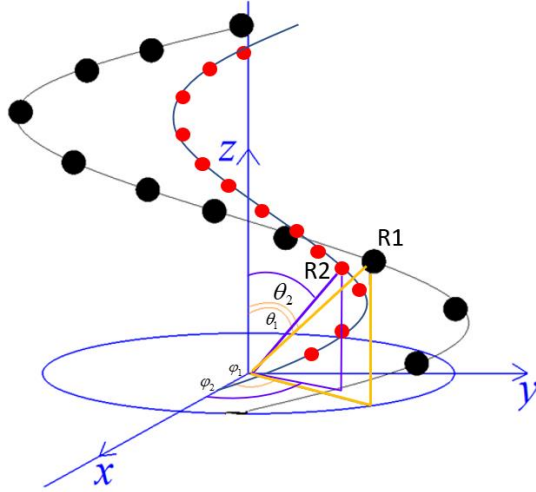


Fig. 3 DNA spirals in the xyz coordinate system. Red indicates internal elements – nitrogenous base atoms, black shows external elements – monosaccharide + phosphoric acid residue.

The calculation of the interference factor of one helix of a molecule was carried out in Ref. [10]. We use the formula obtained in this work:

$$g_N(p) = \left(\frac{N}{2\pi} \right)^2 \left| \int_0^{2\pi} e^{ipR \sin \theta \cos z + ip \cos \theta \times \alpha \times z} dz \right|^2. \quad (3)$$

In the model under consideration, the number of spirals doubles, so the interference factor changes:

$$\left| \sum_{a=0}^N e^{ipR_a} \right|^2 = \left(\frac{1}{2\pi} \right)^2 \left(\left| \int_0^{2\pi} N_1 e^{ipR_1 \sin \theta_1 \cos z + ip \cos \theta_1 \cdot \alpha \cdot z} dz \right|^2 + \right. \quad (4)$$

$$\left. + \left| \int_0^{2\pi} N_2 e^{ipR_2 \sin \theta_2 \cos z + ip \cos \theta_2 \cdot \alpha \cdot z} dz \right|^2 \right),$$

where N – number of elements in the system, $\alpha = 2\pi \cdot CT$ – parameter of the spiral, which depends on its period. Adding two interference factors together is a big simplification. In this case, we consider the system rather roughly, neglecting the interaction of secondary scattering from elements 1 and 2. We believe that the distance between groups of elements inside the same chain. This model takes into account interference between nearby atoms directly involved in hydrogen bonding and phosphodiester bonding. This approach should test the very idea of using a two-element model.

Next, we calculate the scattering spectrum using the form obtained in the work [11]:

$$\frac{d^2W}{d\omega d\Omega_k} = \frac{1}{(2\pi)^2} \frac{f(\omega)}{c^3 \omega} (N_a N_b G(\omega, n, n_0) +$$

$$+ N_a N_b (N_b - 1) F(\omega, n, n_0) +$$

$$N_b^2 Q(\omega, n, n_0) g_N(p)), \quad (5)$$

where $Q(\omega, n, n_0)$, $G(\omega, n, n_0)$, and $F(\omega, n, n_0)$ – atomic form factor, $d\Omega_k$ – solid angle, n – direction of the scattered pulse, n_0 – direction of the incident pulse.

Using Eq. (5), let us simulate the scattering of an ultrashort laser pulse within a simplified model of Eq. (4). The simulation results are presented in Fig. 4, which demonstrates a cross-shaped scattering spectrum, which is similar to classical experiments with X-ray radiation.

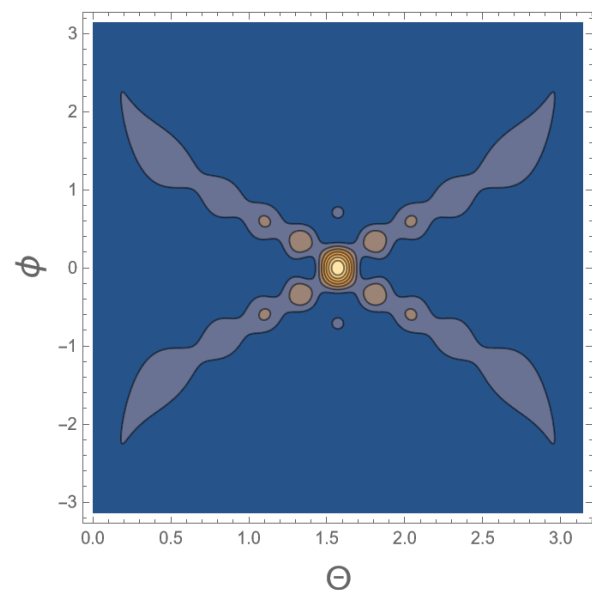


Fig. 4 Contour plot of the scattering spectrum of an ultrashort laser pulse on a simplified model of a molecule: θ – angle between the helix axis and the scattering direction, φ – is the angle between the x-axis and the projection on the plane perpendicular to the helix axis (spherical coordinates).

The simulation was performed in Wolfram Mathematica 13.0. Specified parameters for the simulation: the pulse falls on the system at an angle of 90° . All spectra are normalized to one. A 3D contour plot of the spectrum is shown in Fig. 5, which illustrates the difference between the considered model and the model of paper [10].

As can be seen from Fig. 5(b), the spectrum obtained in Ref. [10] is asymmetric, which indicates that the analytical formula was erroneous. The helix of the DNA molecule has a periodicity, from which it could be assumed that the spectrum of interaction of radiation with it should be symmetrical. After processing of Eq. (3), we obtained a model of the diffraction pattern in Fig. (4), which is symmetrical and similar to X-ray images of a DNA molecule.

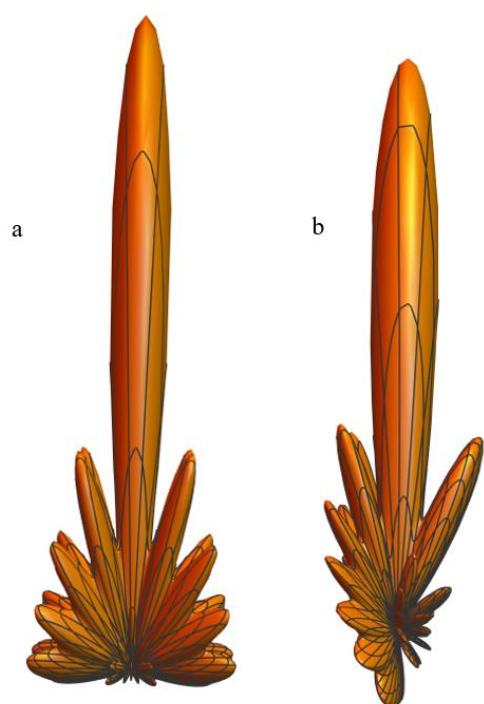


Fig. 5 Contour plot of the scattering spectrum of an ultrashort laser pulse on a simplified model of a molecule: (a) obtained on the basis of calculations of Eq. (4); (b) obtained on the basis of calculations from Ref. [10].

3 Conclusion

The article describes the idea of a new simplified calculation of the interference coefficient for the case of a polyatomic molecular structure when it is irradiated with ultrashort laser pulses. The object of the study is a DNA molecule and a femtosecond laser pulse impinging on it. A simplified model of the system under study is constructed, on the basis of which the formula of the interference coefficient is derived. The results are compared with the previously obtained spectra of the interaction of an ultrashort pulse with a single turn of the molecule without taking into account the internal nitrogenous bases. The simulation results demonstrate that such a way of describing DNA by dividing it into elements is feasible and it is possible to continue working on the theory, complicating it by taking into account repeated emissions.

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Disclosures

The authors declare no conflict of interest.

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Multiparameter Analysis of Statistical Memory Effects in Bioelectric Signals while Performing Cognitive Tasks

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Abstract. In this research, in the framework of Memory Functions Formalism, we study statistical memory effects of electroencephalogram data for two groups of people by performing auto- and cross-correlation analysis. The first group consists of 8 professional musicians; the second group was represented by 11 people without any musical education. Bioelectrical activity signals were recorded during 2 cognitive tasks: perceiving a fragment of musical piece, and perceiving a text read aloud. During autocorrelation analysis, we identify regions of brain cortex, statistical memory effects of signals from which differ the most and use them for the following analysis. During the second stage of work, we identify differences in spectral behavior for both groups and analyze the effects of frequency-phase synchronization. Finally, it is demonstrated that our approach allows detecting differences in the cognitive abilities of people when performing various cognitive task. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: data science; living systems; biomedical data; time series analysis; autocorrelations; cross-correlations; cognition; electroencephalograms.

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1 Introduction

A complex system is a composite object, the parts of which are combined into a single whole according to certain laws or connected with each other by given relationships, because of which such a system acquires new properties that cannot be reduced to the properties of its parts. One of the examples of the most complex system is a human brain. In this work, we analyzed electroencephalogram (EEG) data – one of the methods to record bioelectric human brain activity.

Professional experience and long-term training may lead to a certain change in the activity of human brain cortex, that can be detected by analysis of bioelectric data records [1]. As a result, patterns of brain activity differ between experts and non-experts in different fields. Discrepancies between the biomedical data of professionals and non-professionals can be especially significant during the performance of expert tasks [2, 3].

This paper shows methodology to detect difference in statistical memory effects in signals of two groups of people recorded during two different cognitive tasks.

2 Literature Review

Determining the statistical features of changes in brain activity due to professional activity is not an easy task. In the study of similar data – the analysis of EEG signals when listening to musical fragments by musicians and non-musicians, the statistical parameters of the coherence of EEG signals were studied. The authors found significant changes in long-range θ -coherence in certain areas of the left hemisphere of the brain during learning [4].

In addition, at the moment, machine learning methods are widely used to classify the EEG signals of musicians and non-musicians. Thus, when analyzing the signals recorded during the task of sound perception of musical fragments, several linear and non-linear classifiers were used to classify biomedical data. As a result of a comparative analysis of the resulting models, a neural network model was developed that makes it possible to classify the initial EEG signals with an accuracy of 97.2% [5]. This indicates both the relevance of research on similar data using modern statistical methods, and their high efficiency.

Also, in some works [6] the event-related potential was studied in the framework of the original method of multivariate regression analysis using real-time biomedical data. This methodology allowed the authors to determine the degree of synchronization between the studied experimental data and the sound stimulus, presented both in the form of a melody and in the form of a single-tone sound and noise. As a result, a method for classifying EEG signals was developed, which makes it possible to determine the features of the statistical parameters of biomedical data that manifest themselves when listening to various compositions by people with and without a musical education.

In Ref. [7], when analyzing data, similar to the one studied in our work, the authors applied multivariate statistical analysis to develop a biomedical data classifier for a group of musicians and non-musicians while listening to a musical composition. The authors applied the Principal Component Analysis method and developed an original approach to signal classification when performing the studied expert task with an accuracy of 88%.

Our work is aimed at developing an original method for analyzing the autocorrelations and cross-correlations of the recording of the bioelectrical brain activity of professional musicians and people without musical education. The presented approach is based on one of the modern biomedical methods investigating the dynamics of discrete non-Markovian (idem non-Markov) random processes in complex systems: Memory Functions Formalism (MFF).

The key feature of the proposed approach is considering the high degree of “individuality” of human EEG signals, which manifests itself to an even greater extent in various cognitive and sensory processes. According to the MFF, the individual characteristics of the bioelectrical activity of the human brain, including those under external influences, can be fully considered by performing a simultaneous analysis of autocorrelations and effects of statistical memory, as well as the collective dynamics of neuron ensembles. Under the conditions of cognitive processes and sensory influences, the functioning of various parts of the human cerebral cortex must correspond to some optimal level of such interconnections.

3 The Main Features of the Memory Functions Formalism

MFF is a theoretical methodology developed by the Kazan school of statistical and computational physics as applied to the discrete dynamics of complex non-Hamiltonian systems of various nature [8, 9].

MFF is remarkable because it allows extracting a large number of significant features of the studied time series by performing auto- and cross-correlation analysis and the analysis of statistical memory effects.

In this methodology, the temporal dynamics of an experimentally recorded variable of a complex system,

can be represented as a discrete time series $\{x_j\}$ of a variable X :

$$X = \{x(T), x(T + \tau), x(T + 2\tau), \dots, x(T + (N-1)\tau)\}, \quad (1)$$

where T is the initial time of start from which the recording of experimental variable started, $(N-1)\tau$ is the signal recording time, $\tau = \Delta t$ is the sampling time step.

For a quantitative description of the dynamic properties of the living system under study (correlation dynamics), it is convenient to use the normalized time correlation function (TCF):

$$\begin{aligned} a(t) &= \frac{1}{(N-m)\sigma^2} \sum_{j=0}^{N-m-1} \delta x_j \delta x_{j+m} = \\ &= \frac{1}{(N-m)\sigma^2} \sum_{j=0}^{N-m-1} \delta x(T + j\tau) \delta x(T + (j+m)\tau), \end{aligned} \quad (2)$$

$$t = m\tau, 1 \leq m \leq N-1,$$

where x_j, x_{j+m} are the values of variable X on steps $j, j+m$ correspondingly, δx_j and δx_{j+m} are fluctuations of values x_j, x_{j+m} , σ^2 is the absolute variance of the variable X .

For greater convenience and clarity in terms of the time series' temporal dynamics, we will consider not only the TCF, but, in addition to it, the autocorrelation coefficient calculated as follows:

$$k(x) = \frac{\overline{x_T x_{T+t}} - \bar{x}_T \bar{x}_{T+t}}{\sigma_T \sigma_{T+t}}. \quad (3)$$

Zwanzig-Mori projection operators technique [8, 10] developed in nonequilibrium statistical physics allows obtaining a chain of finite difference equations of non-Markov type for the initial and higher-order memory functions $M_i(t)$ ($i=1, 2, \dots, n$):

$$\begin{aligned} \frac{\Delta a(t)}{\Delta t} &= \lambda_1 a(t) - \tau \Lambda_1 \sum_{j=0}^{m-1} M_1(j\tau) a(t - j\tau), \\ &\dots, \\ \frac{\Delta M_{n-1}(t)}{\Delta t} &= \lambda_n M_{n-1}(t) - \\ &- \tau \Lambda_n \sum_{j=0}^{m-1} M_n(j\tau) M_{n-1}(t - j\tau), \end{aligned} \quad (4)$$

where λ_n are parameters that form the spectrum of eigenvalues of the Liouville quasi-operator, Λ_n are relaxation parameters:

$$\begin{aligned} \lambda_n &= i \frac{\langle \mathbf{w}_{n-1} | \hat{L} | \mathbf{w}_{n-1} \rangle}{\langle | \mathbf{w}_{n-1} |^2 \rangle}, \\ \Lambda_n &= i \frac{\langle \mathbf{w}_{n-1} | \hat{L} | \mathbf{w}_n \rangle}{\langle | \mathbf{w}_{n-1} |^2 \rangle}. \end{aligned} \quad (5)$$

Dynamic orthogonal variables $\mathbf{W}_n$ in Eq. (5) are obtained using the Gram-Schmidt orthogonalization procedure:

$$\langle \mathbf{W}_n, \mathbf{W}_m \rangle = \delta_{n,m} \langle |\mathbf{W}_n|^2 \rangle,$$

where $\delta_{n,m}$ is the Kronecker symbol.

To quantify the effects of statistical memory, the authors proposed a frequency dependence of the non-Markov (idem non-Markovity) parameter [8, 10]:

$$\varepsilon_i(\nu) = \left(\frac{\mu_{i-1}(\nu)}{\mu_i(\nu)} \right)^{\frac{1}{2}}, \quad (6)$$

where the frequency characteristics of $\mu_i(\nu)$ power spectra are determined through the Fourier images of the memory functions $M_i(t)$:

$$\begin{aligned} \mu_0(\nu) &= \left| \Delta t \sum_{j=0}^{N-1} a(t_j) \cos 2\pi \nu t_j \right|^2, \\ \dots, \\ \mu_i(\nu) &= \left| \Delta t \sum_{j=0}^{N-1} M_i(t) \cos 2\pi \nu t_j \right|^2. \end{aligned} \quad (7)$$

Also, we use cross-correlation formalism in our work. The temporal dynamics of an experimentally recorded parameters of a complex system of living nature can be represented as a discrete time series $\{x_j\}$ and $\{y_j\}$ of a variable X, Y :

$$X = \{x(T), x(T + \tau), x(T + 2\tau), \dots, x(T + (N-1)\tau)\}, \quad (8)$$

$$Y = \{y(T), y(T + \tau), y(T + 2\tau), \dots, y(T + (N-1)\tau)\}.$$

By analogy with the autocorrelation formalism, we use cross-correlation coefficient as follows:

$$k(x, y) = \frac{\sum_{t=1}^{T-k} (x_t - \bar{x})(y_t - \bar{y})}{\sqrt{\sum_{t=1}^T (x_t - \bar{x})^2 \sum_{t=1}^T (y_t - \bar{y})^2}}. \quad (9)$$

Besides that, frequency characteristics of power spectra and non-Markov parameter are also defined for the cross-correlation case:

$$\begin{aligned} \mu_0^{XY}(\nu) &= \left| \Delta t \sum_{j=0}^{N-1} c(t_j) \cos 2\pi \nu t_j \right|^2, \\ \dots, \\ \mu_i^{XY}(\nu) &= \left| \Delta t \sum_{j=0}^{N-1} M_i^{XY}(t) \cos 2\pi \nu t_j \right|^2, \end{aligned} \quad (10)$$

$$\varepsilon_i^{XY}(\nu) = \left(\frac{\mu_{i-1}^{XY}(\nu)}{\mu_i^{XY}(\nu)} \right)^{\frac{1}{2}}.$$

In this work we develop an original method for determining the distinctive parameters of the classification of EEG signals recordings of people with different levels of cognitive abilities development. This method is based on the autocorrelation and cross-correlation analysis in the framework of MFF and the study of cross-correlation coefficients.

4 Experimental Data

Experimental data were obtained in the course of the international cooperation program (see Ref. [11], for details).

The data files were records of EEG signals recorded from the cerebral cortex during the performance of various expert tasks for two groups of men:

- 8 musicians (experts), with an average age of 25.7 years, who have been professionally involved in music for at least 5 years;

- 11 people without any musical education (non-musicians or non-experts, control group), with an average age of 25.4 years.

Biomedical data were recorded during the execution of the following tasks by the subjects:

1. The task of perceiving a fragment of a musical piece;

2. The task of perception of the text with neutral content.

The subjects' eyes were closed during all tasks. During the study, the subjects listened to an excerpt from the "French Suite No. 5" by I.S. Bach. Also, the subjects were read aloud the text of a short story in German. The duration of the recording of all time signals was 90 s.

A certain long-term cognitive activity can significantly change the dynamics of the bioelectrical activity of the human brain. Such activity includes, for example, professional activity: the statistical parameters of biomedical data can change significantly during long-term studies in the visual arts [12, 13], musical art [14, 15], etc.

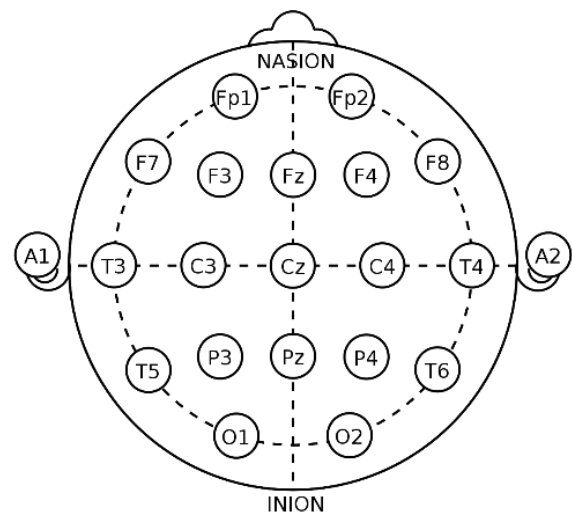


Fig. 1 International scheme of layout of electrodes "10-20%".

Table 1 Mean values and standard deviations of autocorrelation parameters for two groups of people for two tasks.

Parameter	Dataset							
	Musicians, music perception		Non-musicians, music perception		Musicians, text perception		Non-musicians, text perception	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
$\varepsilon_1(0)$	7.73	1.36	9.2	2.12	12.7	2.05	8.2	1.89
$\delta_1(0)$ [8]	380	37	523	49	1158	76	580	63
$k(x)$	0.94	0.05	0.87	0.06	0.82	0.05	0.92	0.07

Recording was carried out with 19 electrodes, located according to the standard international electrode placement system “10–20%” (Fig. 1) with a sampling frequency of 128 Hz. The average signals of the lobes of both ears were used as reference.

5 Analysis of Autocorrelation and Spectral Features of Brain Activity

The analysis was carried out in two stages: in the first stage, the autocorrelation characteristics of all signals were studied. According to the results obtained during autocorrelation analysis, for each person during the performance of each task, non-Markovian parameter $\varepsilon_1(0)$, memory information measure $\delta_1(0)$, and autocorrelation coefficient $k(x)$ were calculated. Next, the average values and standard deviations of the correlation characteristics for two groups of people were calculated (Table 1).

It can be observed that for the two groups of people in both tasks’ correlation features are different. In the case of the task of perceiving a piece of music, the values of the information measure of memory $\delta_1(0)$ and the non-Markov parameter $\varepsilon_1(0)$ are much lower for the representatives of the expert group, while the autocorrelation coefficient $k(x)$ is larger. Respectively, for the task of text perception, the situation is reversed: the values of the signal correlation parameters $\varepsilon_1(0)$, $\delta_1(0)$ (excluding the autocorrelation coefficient $k(x)$) are significantly higher for a group of experts.

As is known, the calculated measures of statistical memory allow dividing the considered processes into three groups: processes with strong memory ($\varepsilon_1(0), \delta_1(0) \rightarrow 1$), processes with moderate (intermediate) memory ($\varepsilon_1(0) \sim 10, \delta_1(0) \sim 100$) and processes with weak memory ($\varepsilon_1(0), \delta_1(0) \rightarrow \infty$). As can be seen from the obtained results, the studied biomedical data show the effects of moderate statistical memory.

Based on the calculated values, a diagram was drawn up showing the electrodes, the signals from which showed the greatest relative differences in statistical parameters for two groups of people (Fig. 2).

Considering the values of the calculated parameters, the electrodes were determined, in which the correlation and spectral features of human brain activity are most pronounced. In the case of the perception of music task

(Fig. 2(a)), the greatest differences were manifested in the signals from the electrodes T_5, P_3 and Fp_1, Fp_2 . In the text perception task, the greatest differences were also found in areas T_3, T_4, T_6, Fp_1 (Fig. 2(b)).

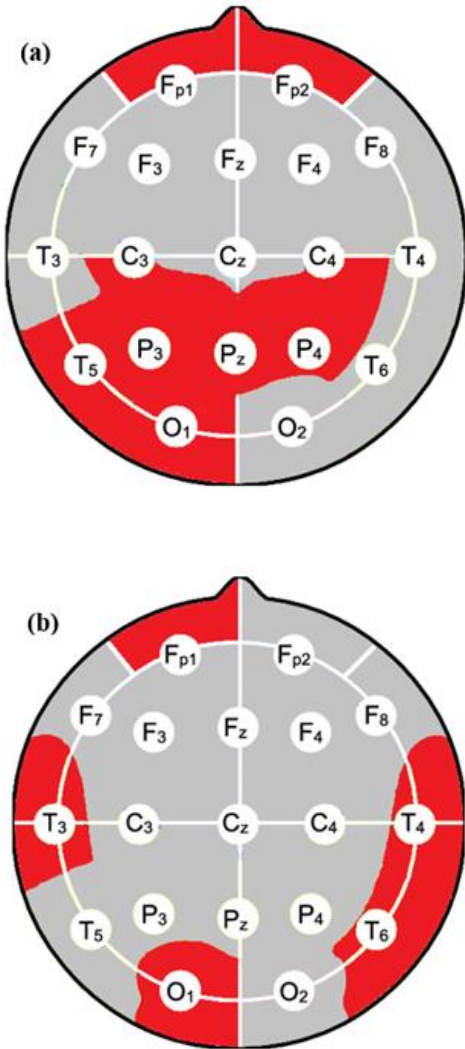


Fig. 2 Electrodes for which the differences in the values of the non-Markov parameters and the autocorrelation coefficient are the most significant; (a) corresponds to the task of music perception, (b) corresponds to the task of text perception.

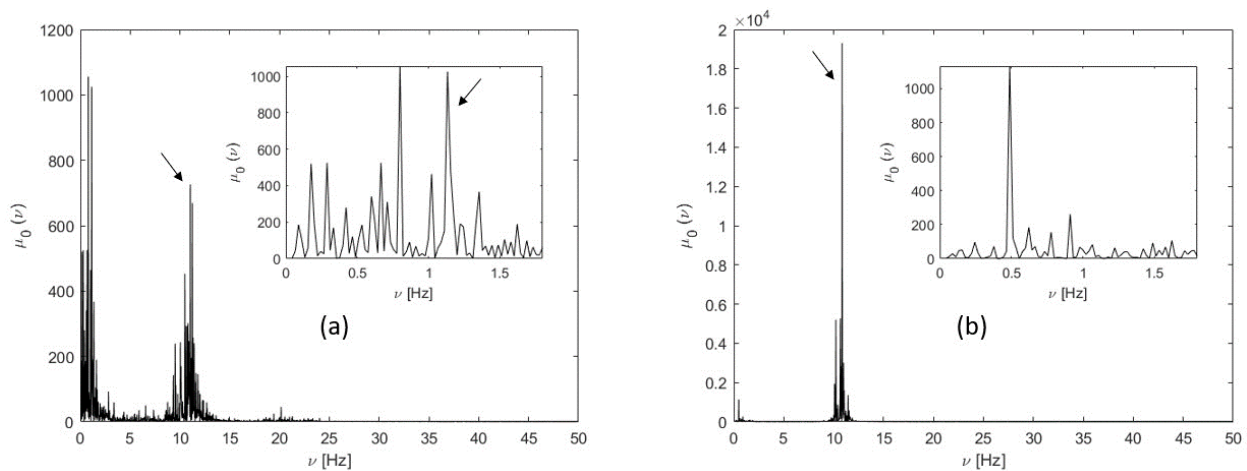


Fig. 3 Power spectra of the statistical memory functions of the signal from the T_5 electrode for the task of music perception for a person from the expert (a) and a control group (b). The arrows mark the peaks with the highest intensity.

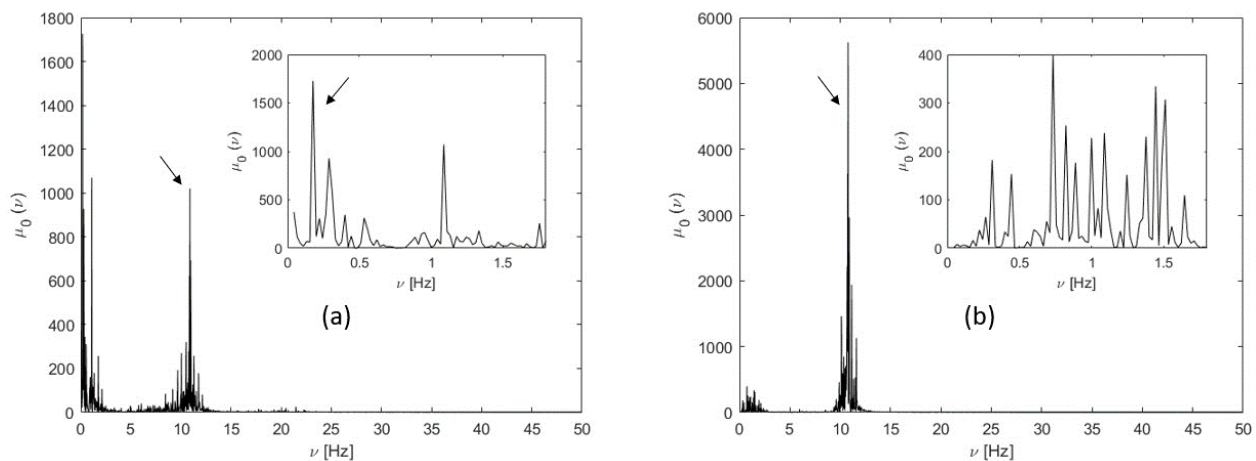


Fig. 4 Power spectra of the statistical memory functions of the signal from the T_3 electrode for the task of text perception for a representative from the expert (a) and a control group (b). The arrows mark the peaks with the highest intensity.

In the case of the task of perceiving music, the intensity of the power spectrum peaks of the control group subject (Fig. 3(b)) is significantly higher than that of a subject from a group of musicians (Fig. 3(a)). Also, for a group of non-musicians, only high-frequency periodic activity (~ 10 Hz) is significantly manifested, and for a group of experts, also low-frequency activity (~ 0.5 Hz) along with the higher periodic activity (~ 8 Hz).

In the case of the text perception task, the maximum intensity of the power spectrum peaks of non-musicians (Fig. 4(b)) turned out to be significantly greater than the intensity of the power spectrum peaks of a subject from a group of musicians (Fig. 4(a)). Also, for the control group, high-frequency periodic activity (~ 10 Hz) is significantly manifested, and for the representative of the group of musicians, in addition, low-frequency activity (~ 0.5 Hz) is also observed.

For each task, the experimental data of one person from each group with non-Markovian parameters and memory information measures close to the group

averages were selected for subsequent cross-correlation analysis.

6 Analysis of Cross-Correlation and Spectral Features of Brain Activity

For a more complete identification of the specific features of the studied biomedical data within the framework of the MFF, in addition to the above analysis of autocorrelations, a cross-correlation analysis was carried out. Cross-correlation coefficients were calculated and averaged over the study groups. For visualization, the relative values of the cross-correlation coefficients were calculated and presented in the form of a 3D graphs (Fig. 5 and Fig. 6).

Comparative analysis shows that the difference between the experts and the control group manifest itself to the greatest extent in the task of perceiving a piece of music. In addition, the absolute value of the ratio of cross-correlation coefficients is maximum for pairs of electrodes F_7-P_z , Fp_2-P_z , F_8-C_4 , Fp_1-T_6 when

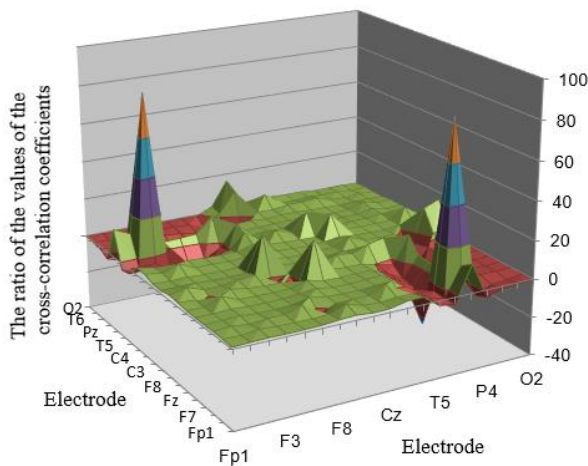


Fig. 5 The ratio of the cross-correlation coefficient of experts to the cross-correlation coefficient of the control group depending on the choice of interacting electrodes in the task of music perception.

performing tasks of music perception (Fig. 5); for time series recorded by pairs of electrodes F_8-O_2 , Fp_2-O_2 , Fp_1-O_1 , Fp_1-T_6 in the task of text perception (Fig. 6). It can be seen that the greatest differences appear when considering the signals from the electrodes in the frontal and occipital regions of brain.

The identification of such correlation features is necessary for further analysis to reduce the number of considered electrode interactions.

Further, a cross-correlation analysis of typical representatives of their groups was carried out according to the combination of Fp_1-T_6 electrodes. During the analysis, graphs of the power spectra of the statistical memory functions were obtained (Fig. 7 and Fig. 8).

As a result, it was found that, on average, the intensity of the power spectrum peaks is significantly higher for the control group (Fig. 7(b) and Fig. 8(b))

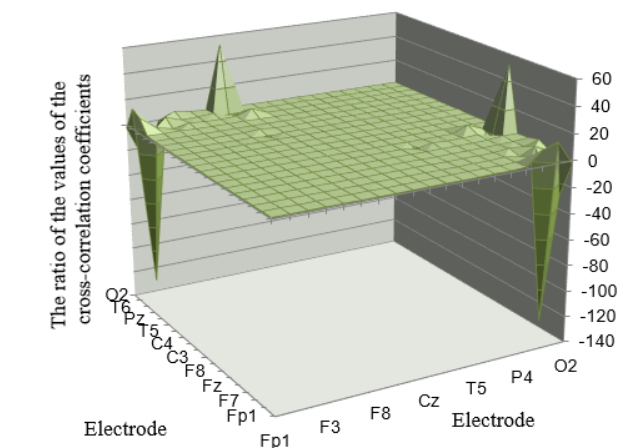
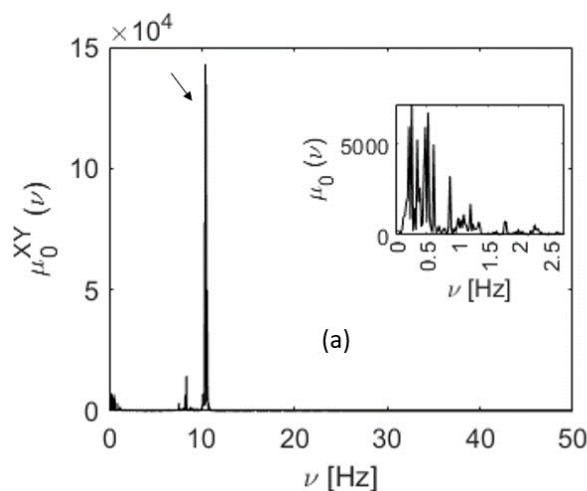


Fig. 6 The ratio of the cross-correlation coefficient of experts to the cross-correlation coefficient of the control group depending on the choice of interacting electrodes in the task of text perception.

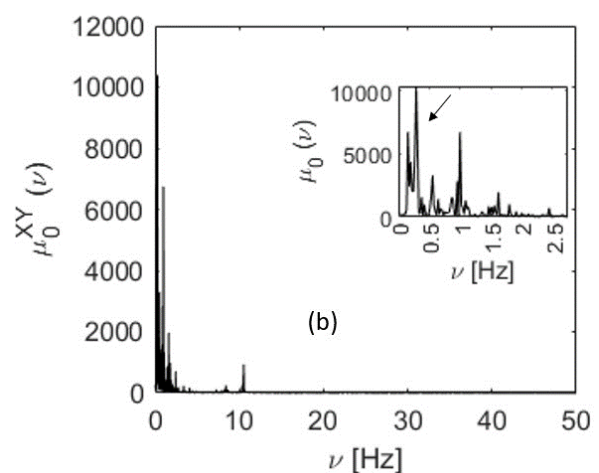


Fig. 7 Power spectra of the statistical memory functions of the signal from the Fp_1-T_6 electrodes for the task of music perception for a subject from the expert (a) and control group (b). The arrows mark the peaks with the highest intensity.

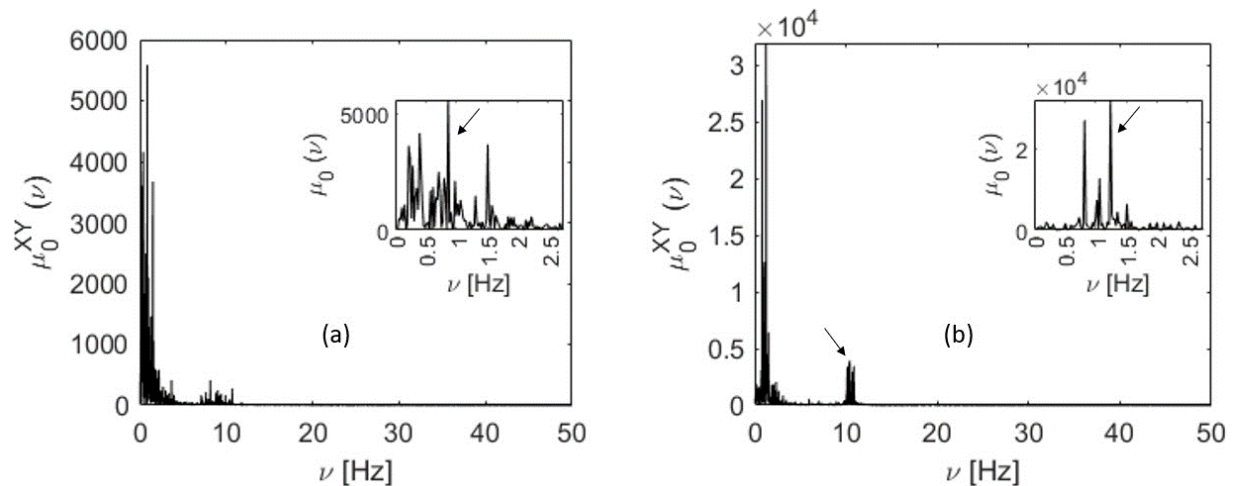


Fig. 8 Power spectra of the statistical memory functions of the signal from the Fp_1-T_6 electrodes for the task of text perception for a subject from the expert (a) and control group (b). The arrows mark the peaks with the highest intensity.

7 Conclusion

In this work, an original method for analyzing auto- and cross-correlations of human biomedical data is being developed. The approach is based on the finite-difference analogue of the Zwanzig-Mori equations intended for studying the discrete stochastic dynamics of complex systems.

In the framework of Memory Functions Formalism, we discovered that the dynamics of biomedical parameters is characterized by more random nature for non-experts when listening to a fragment of a musical work; for experts – while listening to the text. This indicates the occurrence of periodic processes in their biomedical parameters, and, consequently, a greater degree of self-consistency of their signals when performing these tasks.

Also, while performing all tasks in the biomedical data of the representatives of the expert group, in contrast to the representatives of the control group, high-frequency periodic activity was manifested to a much greater extent.

Moreover, when analyzing the effects of frequency-phase synchronization, significant differences were found in the degree of correlation of biomedical signals recorded in remote areas of the cerebral cortex for two groups of subjects.

In conclusion, it should be noted that the proposed MFF approach, as a tool that is sensitive to identifying

the features of the measured signals during their parameterization, can be used in a comprehensive analysis of the phenomenon of high individuality of biomedical data. At the same time, certain ranges of changing resonant frequencies and the limits of permissible changes in the values of MFF parameters with a set of “standard” influences are considered as “indicators of individuality” of each organism.

Our further research into the search for statistical patterns in the dynamics of human EEG signals during various cognitive processes will be associated with other methods of statistical analysis, such as Flicker-Noise Spectroscopy [17, 18], and machine learning [19, 20]. Within the framework of Flicker-Noise Spectroscopy, additional possibilities appear for calculating information parameters that characterize the chaotic components of the studied signals in different frequency ranges, as well as for studying frequency-phase correspondences.

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Disclosures

The authors declare no conflict of interest.

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Potential Thermal Effect of Stimulating Brain Tissue during Low Level Laser Therapy

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Abstract. Low level laser therapy (LLLT) is a promising and noninvasive technique in treating a multitude of medical conditions by activating healing and cell regeneration. It is also used to stimulate the brain function. The aim of this study is to investigate the Potential Thermal Effect of LLLT during stimulation. LLLT is characterized by low-intensity treatment. However, what is the dose of intensity required to stimulate the brain without a possible thermal effect. To address this, a simulation model was proposed and implemented using Finite Element Analysis within the COMSOL Multiphysics software package. This approach aims to determine the optimal combination of energy density and irradiation time that would yield the most effective enhancement of cell activity in the brain. The best power density is 166 mW/cm² (joule density 20 J/cm²) and 2 min exposure is enough to stimulate the brain when applying 808 nm with optic cap that gives a laser spot size of 3 cm². The determination of optimal parameters is imperative in the context of brain activation. It is crucial to ensure that the tissue temperature does not exceed 0.5 °C, which is the permissible temperature limit for effective stimulation. The findings will provide valuable insights into the optimization of LLLT protocols, thereby establishing a foundation for its safe and effective application in therapeutic settings. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: stimulation; Low Level Laser Therapy; COMSOL multiphysics; brain; thermal effect.

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1 Introduction

Low level laser therapy (LLLT) is a simple, safe, noninvasive treatment that uses low intensity of laser light to treat a multitude of conditions that require stimulation of healing or relief of pain [1] and inflammation [2], restoration of function [3], and rejuvenation and brain health disorders [4].

The basic concept of LLLT is the delivery of therapeutic doses of laser energy to affected tissues at an energy level that is much lower than the threshold required for thermal damage but sufficient to cause tissue reaction [5]. Where visible or near infrared (NIR) lasers (600–1064 nm) are typically used with an irradiation time

of a few seconds to minutes or sometimes even more, in the low power range of (1–500 mW) [5], and an increase in temperature up to 0.5 °C is enough to cause changes in cell function [6].

Despite many the positive results, still there is some controversy about the effectiveness of LLLT, this is because the mechanism of its interaction is not well understood, beside that the optimal parameters have not been systematically determined yet [7].

LLLT has been shown to increase blood flow and oxygenation in various parts of the body, including those areas where it was applied. This may help improve cellular function and increase energy production, which

is the adenosine triphosphate ATP within cells, and thus stimulate the activity of neurons [8].

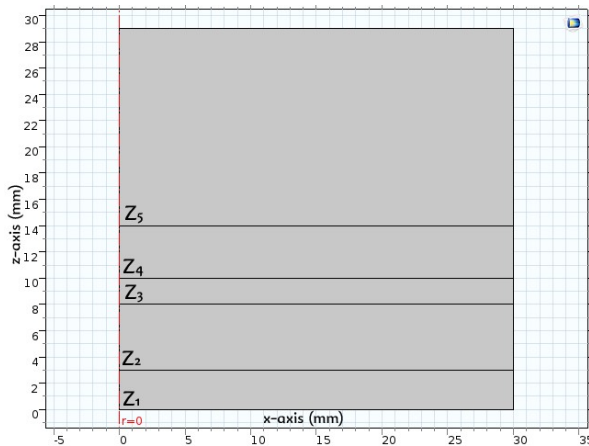


Fig. 1 Brain layers model, z_1 is a scalp, z_2 is a skull, z_3 is a cerebrospinal fluid (CSF), z_4 is a gray matter, and z_5 is a white matter.

Table 1 Typical thickness of brain tissue layers.

Tissue layers	Thickness (mm)	Refractive index n
Scalp	3	1.33
Skull	5	1.34
Cerebrospinal fluid (CSF)	2	1.37
Gray Matter	4	1.39
White matter	20	1.40

Lampl [9, 10] and Hashmi et al. [11] suggested that the utilization of transcranial laser stimulation with low-energy lasers within the near-infrared wavelengths has the potential to modulate brain functions and elicit neurotherapeutic effects. Barrett and Gonzalez-Lima [12] conducted the initial controlled study demonstrating that transcranial laser stimulation enhances cognitive and emotional brain functions in humans. The researches by Rojas and Gonzalez-Lima [13–15] and Chung et al. [16] have provided valuable insights into the historical progression of this field as well as the underlying principles and applications of LLLT in enhancing cortical metabolic capacity and memory retention even in individuals with Alzheimer's disease.

Table 2 Thermal properties of the simulation model.

Tissues	Heat Conductivity $W/(m \cdot K)$	Density kg/m^3	Specific Heat Capacity $J/(kg \cdot K)$	Metabolic Heat (W/m^3)
Scalp	0.342	1100	3391	363
Skull	1.15	1990	1313	70
CSF	0.64	1000	4096	0
Gray Matter	0.5	1080	3696	16700
White matter	0.5	1080	3583	4175

The temperature of biological tissue when subjected to laser stimulation is not only dependent on the laser parameters but also on the thermal attitude of the tissue towards the wavelength of applied laser as well. Consequently, the thermal response of the tissue tends to vary even when exposed to the same parameters of the laser.

Heating the scalp and skull also has a significant impact on the path and position of thermal propagation within the layers of brain tissue. Hence, it is essential to consider the limitations associated with LLLT applied on the human brain due to the possible thermal effects.

Many studies have attempted to model thermal changes in biological tissues under laser irradiation [17, 18]. Simulation are usually done by studying the light diffusion and temperature changes in biological tissue that occur after the absorption of the light energy of laser [19].

The Pennes' bio-heat equation to study the temperature distribution in biological tissues, is an important step in simulating laser-tissues interaction [20, 21]. It is the most famous equation, and it is considered good because it deals with the effects of blood perfusion and metabolism on the biological tissue, which is so important in stimulation, in addition to being simple and accurate, thus it was used to study the heat distribution in the brain layers in our simulation model [18, 21, 22].

The importance of the presented work lies in exploring the potential thermal effect that occurs during neural stimulation, which has not been reported previously, where the parameters used in LLLT has not determined as well. Laser 808 nm was chosen because it is the widely used in stimulation. This study can be used as a reference for other researchers who want to experiment with LLLT in their studies.

2 Modeling Method

The aim of this study was to analyze the temperature distribution of the human brain tissue under variable near-infrared laser irradiation in order to estimate the thermal range, which is related to the activation of neurons. The optimal LLLT parameters such as power density (energy density) and treatment time were investigated during applying laser 808 nm with spot size of 3 cm^2 . Using a model based on finite element method FEM implemented on COMSOL Multiphysics 5.0 software and Pennes' bio-heat transfer equation.

Table 3 Optical parameters of brain tissue.

Tissues	Absorption coefficient, μ_a (1/m)	Scattering coefficient, μ_s (1/m)
Scalp	18	11818
Skull	16	10909
CSF	1	66
Gray Matter	25	7700
White matter	5	36450

Table 4 Effect of power density and treatment time on the tissue temperature distribution.

Power (mW)	500	400	300	200	100
Laser spot size (cm ²)	3	3	3	3	3
Power density (mW/cm ²)	166	133	100	66	33
Irradiation time (min)	Tissue temperature (°C)				
1	37.39	37.33	37.27	37.25	37.22
2	37.59	37.48	37.38	37.29	37.29
4	37.84	37.70	37.55	37.39	37.35
5	37.94	37.77	37.61	37.44	37.37
10	38.22	38.00	37.79	37.58	37.42
15	38.32	38.09	37.86	37.63	37.44
20	38.39	38.14	37.90	37.65	37.45

2.1 Bioheat Transfer Equation

The heat transfer in tissue is a complex process and the main sources of heat transfer are via radiation, air conduction and convection. In addition, blood flow also affects heat transfer. Therefore, the Pennes' bioheat equation is used to calculate the temperature of the tissue irradiated by laser exposure [20, 23, 24]:

$$\delta_{ts} \rho C \frac{\partial T}{\partial t} + \nabla(-K \nabla T) = \rho_b C_b \omega_b (T_b - T) + Q_{met} + Q_{Laser}, \quad (1)$$

where δ_{ts} is a time ratio (the default value is 1), ρ is a density of tissue (kg/m³), C is a specific heat of tissue (J/(kg·K)), T is a temperature (°C), K is a thermal conductivity of tissue (W/(m·K)), ρ_b is a density of blood (kg/m), C_b is a specific heat of blood (J/(kg·K)), ω_b is a volumetric perfusion rate (kg/(s·m)), T_b is a temperature of arterial blood (°C), Q_{met} is a metabolic heat source (W/m³), Q_{Laser} is a laser heat source (W/m³).

2.2 LLLT Propagation Calculation

While a fraction of laser light is absorbed by the tissue, the remaining light will be scattered, reflected or refracted. Here, we suppose that the light source is Gaussian beam and we can define Q_{Laser} in Eq. (1) as follows [19, 22, 24]:

$$Q_{Laser} = \mu_a [(1-R) \frac{2P}{\pi \omega_0^2} \exp(\frac{-2r^2}{\omega_0^2}) \exp(-\mu_t z) f(t)], \quad (2)$$

where μ_a is an absorption coefficient (1/m), R is a reflectance at normal incidence, P is a laser power (W), ω_0 is a beam radius at irradiation surface (m), $\mu_t = \mu_a + \mu_s$ is an attenuation coefficient of tissue (1/m), μ_s is a scattering coefficient (1/m), $f(t)$ is a time function.

While the brain consists of multiple layers, each one has a different optical parameter value. The depth coordinates of each layer are defined as z_1, z_2, z_3, z_4 , and z_5 , which represents scalp, skull, cerebrospinal fluid (CSF), gray matter (adipose tissue), white matter (granular tissue) respectively as demonstrated in Fig. 1. Thus the heat source for the first and second layers as follows:

The heat source of the Scalp layer (first one) is:

$$Q_{Scalp} = \mu_{a1} \cdot [(1-R) \frac{2P}{\pi \omega_0^2} \exp(\frac{-2r^2}{\omega_0^2}) \cdot \exp(-\mu_{s1} z - \mu_{a1} z) \cdot f(t)], \quad (3)$$

The heat source of the skull layer (second one) is:

$$Q_{Skull} = \mu_{a2} \cdot [\frac{2P}{\pi \omega_0^2} \exp(\frac{-2r^2}{\omega_0^2}) \cdot \exp(-\mu_{s1} z_1 - \mu_{s2}(z - z_1) - \mu_{a1} z_1 - \mu_{a2}(z - z_1)) \cdot f(t)], \quad (4)$$

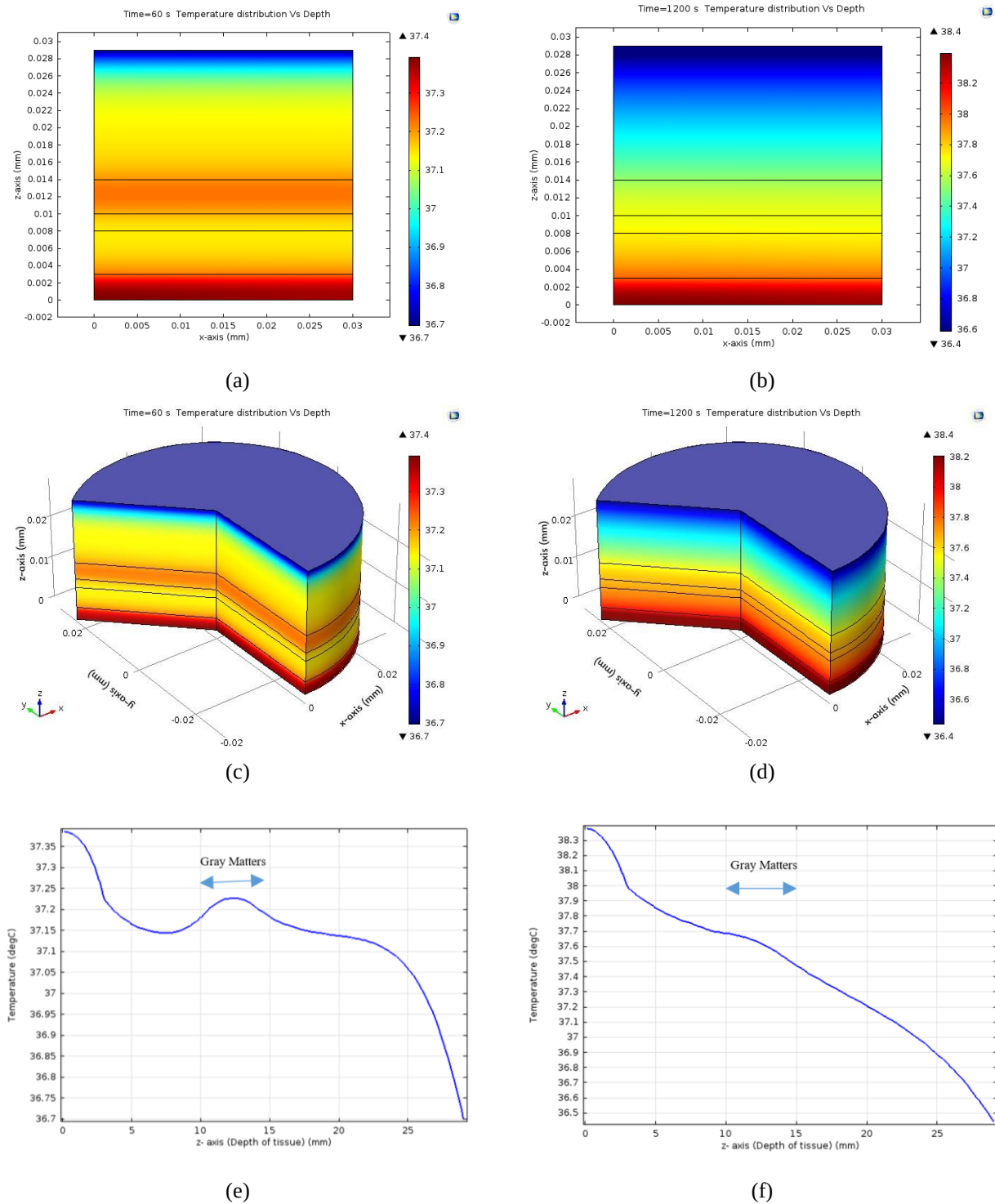


Fig. 2 2D temperature distribution for power density 166 mW/cm^2 at (a) $t = 60 \text{ s}$ and (b) 1200 s ; temperature distribution in a cylinder model at (c) 60 s and (d) 1200 s ; temperature versus depth of tissue (e) at 60 s , and (f) 1200 s .

For the rest of the layers, the heat source equations will be written in the same way, taking into account the optical parameters and thickness of each layer.

Since the heat source changes with time, it should be multiplied by a time function

$$f(t) = \exp\left(-4 \cdot \frac{(t - \tau)^2}{\tau^2}\right), \quad (5)$$

where t is the calculation time (s), and τ is the irradiation time (s).

2.3 Simulation Model

The simulation is based on 2D model mimic the human brain using FEM software COMSOL Multiphysics 5.0.

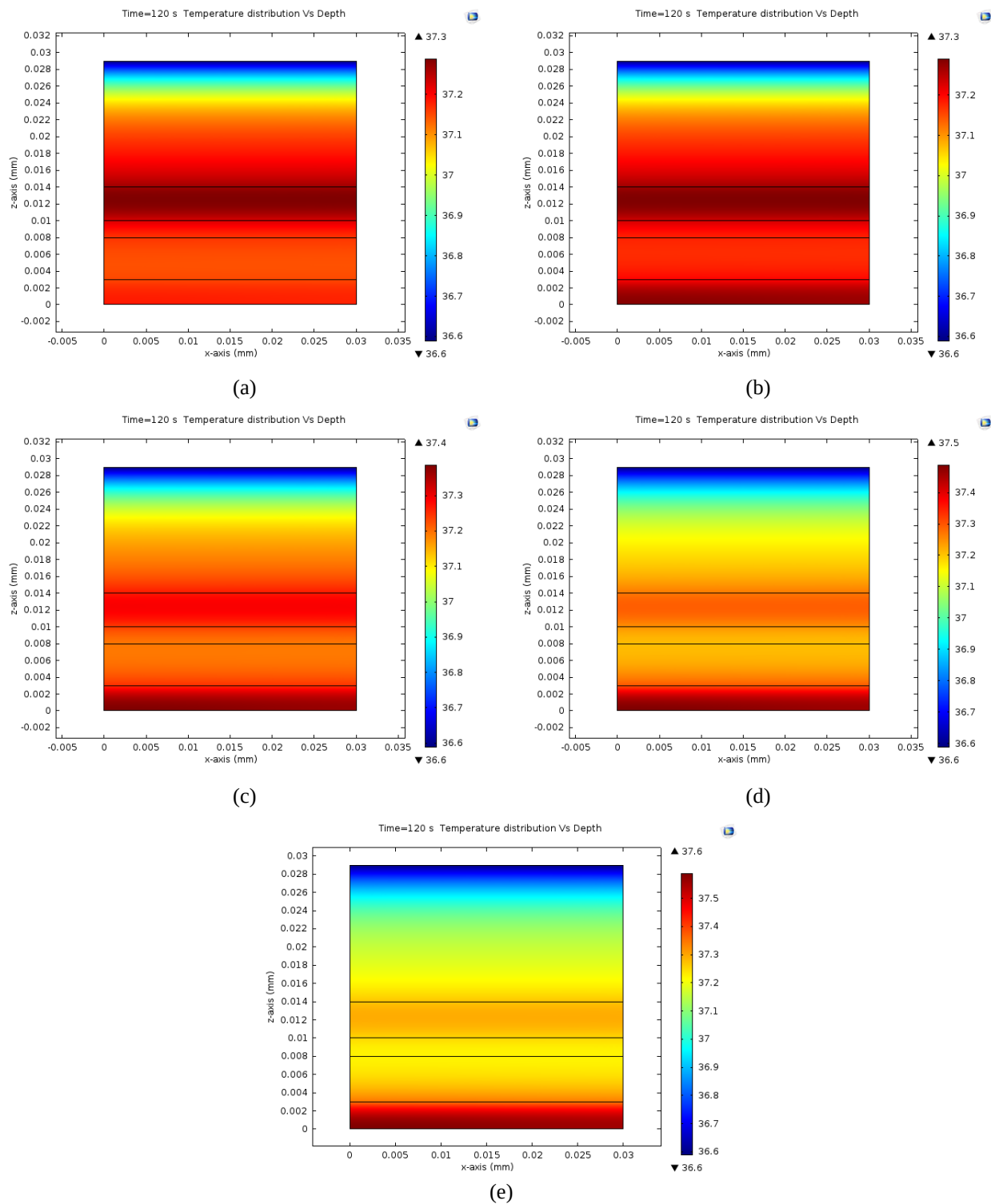


Fig. 3 2D temperature distribution at $t = 120$ s for different power density: (a) 33 mW/cm^2 , (b) 66 mW/cm^2 , (c) 100 mW/cm^2 , (d) 133 mW/cm^2 , and (e) 166 mW/cm^2 .

To increase the computational speed process, the 2D axisymmetric coordinate model was designed, and then revolved into a cylindrical shape, where r -axis represents the radius of the cylinder and z -axis represents the depth in brain tissue inhomogeneous multilayer. Fig. 1 indicates the geometry model, which consists of five layers z_1 , z_2 , z_3 , z_4 , and z_5 are scalp, skull, CSF, gray

matter (adipose tissue), and white matter (granular tissue) respectively [25, 26]. The bioheat equation, Eq. (1) was used to calculate the heat transfer from a continuous-wave laser, Gaussian beam with a wavelength 808 nm (Konftec Laser, Klas DX, for LLLT), and optical cap that gives a laser spot size of 3 cm^2 . The thickness of each layer and refractive index n is given in Table 1 [26, 27].

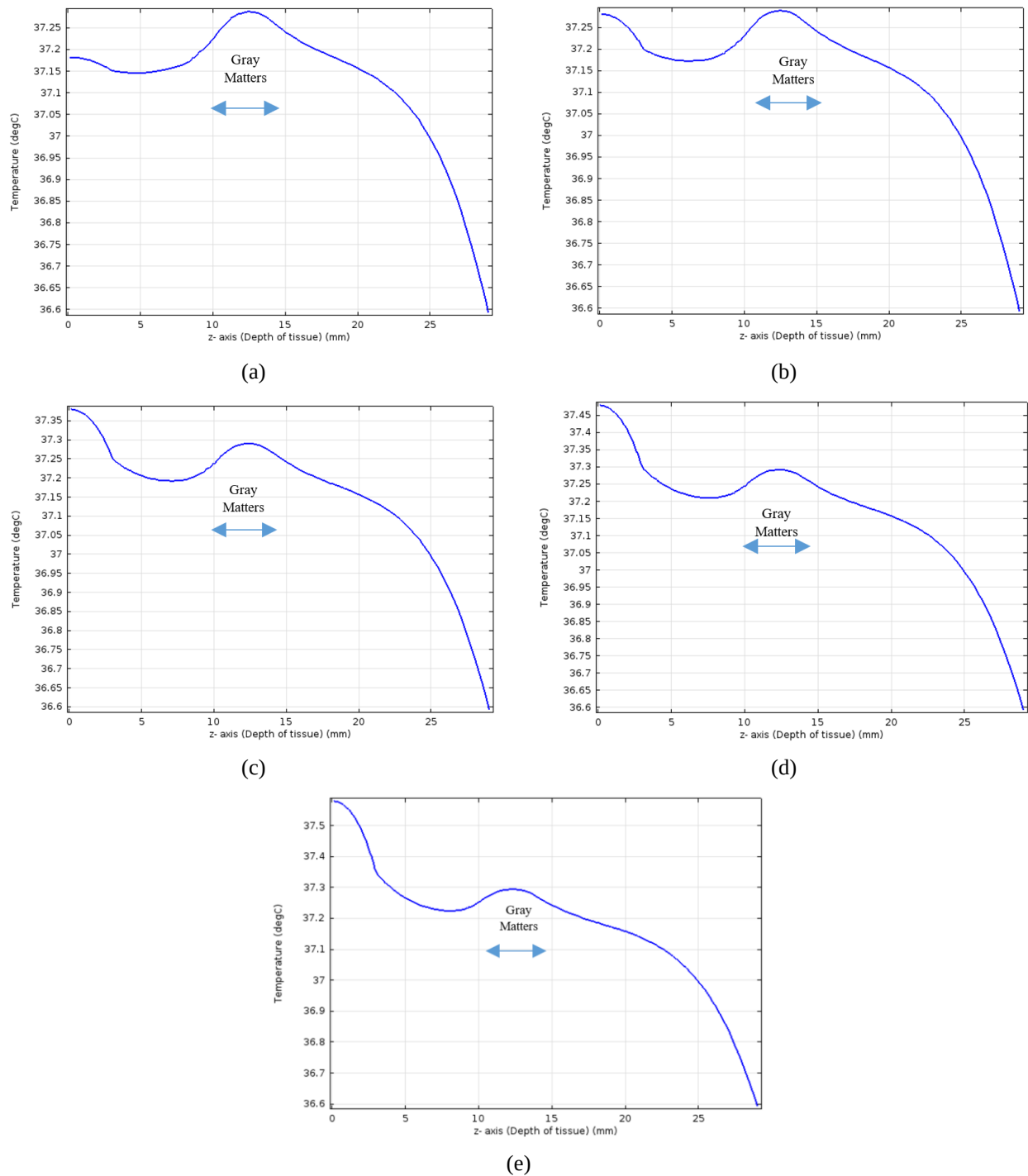


Fig. 4 Temperature versus depth of tissue (z) at $t = 120$ s for power density (a) 33 mW/cm^2 , (b) $P = 66 \text{ mW/cm}^2$, (c) 100 mW/cm^2 , (d) 133 mW/cm^2 , and (e) 166 mW/cm^2 .

The thermal properties and optical parameters which used are summarized in Table 2 [28–30] and Table 3 [31–35] respectively. The blood density is 1060 kg/m^3 , the water content in blood is about 80% [31]. The blood heat capacity is $3770 \text{ J/(kg}\cdot\text{K)}$ [32] and the arterial blood temperature is supposed to be 37°C .

In Eq. (2), the reflectance $R = \frac{(n-1)^2}{(n+1)^2}$, and n is the

refractive index of the tissue which are summarized in Table 1 [26, 27].

In this COMSOL model, the initial and four boundary conditions are defined as follows:

1) Line of axial symmetry is set to be thermally insulated;

Table 5 Best power density and irradiation time for treatment by low level laser therapy (LLLT).

Power (mW)	500	400	300	200	100
Laser spot size (cm ²)	3	3	3	3	3
Power density (mW/cm ²)	166	133	100	66	33
Irradiation time (min)	2	2	4	10	20
Joule density (J/cm ²)	20	16	24	40	40
Tissue Temperature (°C)	37.59	37.48	37.55	37.58	37.45

2) The scalp surface is set to be a natural convection boundary with the surrounding air $q_{conv} = h(T_{inf} - T)$ [36]. Where q_{conv} is the heat flux due to convection, and T is the surface scalp temperature, 37.1 °C; h is the heat transfer coefficient, 10 W/(m²·K) and T_{inf} is the external temperature, 25 °C;

3) Boundaries between inner layers are set to be continuity; and we supposed the initial temperature of other boundaries, 37.2 °C [36].

3 Results and Discussions

The finite element simulation results give temperature distribution of the brain tissue during stimulation process at different laser power density (energy density) and time irradiation, are displayed in Table 4, Fig. 2, Fig. 3, Fig. 4, and Fig. 5.

Table 4 shows that the higher laser power density and the longer time irradiation, the temperature of brain tissue increased, this rise could reach more than 0.5 °C for a long time, so we should be careful even when we are dealing with LLLT. The appropriate stimulation time for brain tissue should not exceed two minutes (120 s) at 166 mW/cm², 20 J/cm², but we can apply a maximum power density of 100 mW/cm², 24 J/cm² so the stimulation time can be increased to 4 min (240 s) without happening any undesired heat effect. It also appears, when laser power density is increased by keeping the irradiation time constant, the upper temperature of the brain is increasing linearly, this is consistent with Vitkaya's simulation outcome [18].

Table 5 summarizes the best power density, joule density, and irradiation time for treatment by LLLT safely.

Fig. 2, demonstrates overheating in all layers of the brain, while the red color gradations express where the most heating occurs. Fig. 2(a) represents two dimensions, and Fig. 2(b) illustrates after revolved in a cylindrical shape, the greatest rise appears when applying laser power density of 166 mW/cm² (20 J/cm²) and the stimulation time 60 and 1200 s, the temperature of the scalp, reaches 37.39 °C and 38.39 °C, respectively. Fig. 2(c) represents the changes in temperature within the tissue depth (z).

Fig. 3 shows that although the cerebrospinal fluid is located before the gray matter, the temperature of CSF is quite lower than the temperature of the gray matter, as it

is shown in the simulation how the temperature of the scalp increases during stimulation and then decreases in the area of the skull and CSF and returns to collect energy again in the gray area, a small amount of laser energy can be scattered in the white matter, and this is clearly visible when a small amount of time and power density, such as 33 and 66 mW/cm² at 60 and 120 s respectively. This happens because gray matter has a higher absorption of the 808 nm laser compared to CSF and white matter. We also note that when the intensity (power density) increases, most of the heat will accumulate in the first scalp layer, thus if we want to stimulate the inner layers, we should apply low intensity and time such as (33 and 66 mW/cm²) at (60 and 120 s).

Fig. 4 illustrates the temperature versus the irradiation time, and another peak is visibly in the gray matter, especially in 33 and 66 mW/cm² at 60 and 120 s as we mentioned that before.

4 Conclusions

Stimulation using LLLT in the field of red to near-infrared light energy has gained a great interest in recent years as a new and safety therapeutic application in neurology.

We found that the thermal effects are negligible (<0.5 °C) and below the human thermal pain threshold when we use very low power (100, 200 mW), power density (33, 66 mW/cm²), and low time (60, 120 s). Besides, low intensities tend to work better than higher intensities for the same wavelength, and it will affect the inner layers as well. However, doses delivered with an 808 nm laser (about 500 mW, Power density 166 mW/cm²) even it is rather low are able to significantly increase tissue temperature.

Therefore, there must be a mechanism to verify the correctness of the steps involved in determining the appropriate dose and time for stimulation before applying it clinically, and this is what the simulation achieves in this study.

Disclosures

The authors declare no conflict of interest.

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VGG Convolutional Neural Network Classification of Hyperspectral Images of Skin Neoplasms

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Abstract. The article is devoted to the problem of early diagnosis of cancer. In last five years, various optical methods have been increasingly used to study biological tissues. This study aims to investigate the capability of a convolutional neural network classifier to diagnose skin cancers. The article analyzes hyperspectral images of malignant melanoma and pigmented nevus. A hyperspectral image classifier based on a deep learning neural network was developed. The results show a classification accuracy of diagnose prediction (on test data) at the level of 95%, which demonstrates the possibility of using machine learning for the classification of hyperspectral images of skin diseases. The results of the study can be applied in medical decision-making systems. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: hyperspectral imaging; malignant melanoma; classification; medical diagnostics; neural network; pigmented nevus; skin cancer.

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1 Introduction

Skin cancer is one of the most common cancers in the world. According to the World Health Organization, 1,200,000 new cases of skin cancer have been identified in 2020 [1]. The vast majority of skin cancer deaths in recent decades have been caused by malignant melanoma (MM), despite the fact that it accounts for only one percent of all skin cancer cases [2]. MM is a dangerous, rare and deadly type of skin cancer. It is possible to increase the patient's chances of survival with skin cancer only through early diagnosis. However, the main difficulty in the qualitative detection of MM tumors is its external similarity with such a benign neoplasm as a pigmented nevus (PN).

As a gold standard, doctors usually use biopsy to diagnose skin cancer. This procedure takes a sample from a suspected skin lesion for medical examination whether it is cancerous or not. However, this procedure takes a lot of time and is also painful for the patient. Besides, the diagnosis of the skin can be held by dermatologists with using dermatoscope cameras. Difficulties in diagnosing skin cancers by general practitioners are associated with the difficulty of interpreting clinical signs and the inability to distinguish MM from benign neoplasms such as PN. In order to avoid unnecessary surgical procedures due to the uncertainty of current diagnoses and overcome

the limitations of conventional imaging, new methods to improve skin cancer diagnosis need to be explored.

In last decade, optical diagnostic methods are gaining popularity. One of these methods is hyperspectral imaging (HSI) [3, 4], which allows not only obtain optical images of the studied areas, but also spectral data on the scattering or absorption of radiation by this area. This imaging technique combines digital imaging with spectroscopy techniques to provide improved spectral properties of the captured area within and beyond the visible range of the electromagnetic spectrum.

A hyperspectral image is a three-dimensional data array (hypercube) consisting of successive images at different wavelengths [5]. This image is obtained by photographing the object at different wavelengths. In such image, each pixel contains the so-called spectral signature of a material or substance, located in the corresponding spatial coordinates. Quantitative information about tissue physiology can be extracted using spectral analysis [6]. HSI provides information on the intensity of reflected light at different wavelengths at each point on the sample surface. To solve the classification problems, it is necessary to use different ranges of registered two-dimensional images that form a hypercube.

HSI for skin pathology studies solves two problems at once: (1) examination of the skin surface to detect the

boundaries of lesions and (2) examination of the morphological and chemical properties of the skin to detect and identify the type of tumor. This technology improves the accuracy of diagnosing skin pathologies, taking into account the physiological characteristics of the patient, as well as the composite and morphological features of the skin surface. The effectiveness of the applied method was demonstrated in the study by Sherendak et al. Authors implemented manual processing of hyperspectral images [7]. Based on the results of this study, it can be concluded that even in the absence of a large number of samples and additional pre-processing of the training set, it is possible to obtain a high accuracy of classification of malignant skin lesions.

Machine learning has been revolutionized in recent decades by deep learning. It is considered to be one of the most difficult areas of machine learning related to artificial neural network algorithms. These algorithms were largely inspired by the functions and structure of the human brain. Deep learning methods can be applied in various fields such as speech recognition [8] and pattern recognition [9]. In these applications, deep learning systems have achieved impressive results by comparison to other classical machine learning methods.

The advantage of using a neural network classifier for hyperspectral images is the ability to use the same architecture and the same code to diagnose various types of diseases and pathologies after performing the necessary training. This means that with successful training, the artificial neural network will make it possible to obtain the correct diagnosis based on data that was not in the training set.

Automated deep learning algorithms based on convolutional neural network (CNN) have achieved remarkable performance in the detection, segmentation, and classification operations of medical imaging. Lequan et al. [10] proposed a very deep CNN for melanoma detection. A fully convolutional residual network (FCRN) having 16 residual blocks was used in the segmentation process to improve performance. The proposed technique used an average of both support vector machine and softmax classifier for classification. It showed 85.5% accuracy in melanoma classification with segmentation and 82.8% without segmentation. DeVries and Ramachandram [11] proposed a multi-scale CNN using an Inception-V3 deep neural network that was trained on an ImageNet dataset. For skin cancer classification, the pre-trained Inception-V3 was further fine-tuned on two resolution scales of input lesion images: coarse-scale and finer scale. The coarse-scale was used to capture shape characteristics as well as overall contextual information of lesions. In contrast, the finer scale gathered textual detail of lesion for differentiation between various types of skin lesions [12].

This paper considers hyperspectral images of MM and PN based on the architecture of a five-block CNN neural network. The aim of the study is to investigate the capabilities of a neural network for the classification of hyperspectral images of skin pathologies.

2 Materials and Methods

To record hyperspectral images *in vivo*, an acousto-optical hyperspectral camera was used, which makes it possible to obtain an image of the area under study at an arbitrarily set wavelength in the range of 440–750 nm (Fig. 1). In this case, the spectral resolution is $\delta\lambda = 2.5$ nm (at $\lambda = 633$ nm), and the spatial resolution is 0.14 mm. A feature of the installation is double sequential filtration. It provides almost complete compensation of spectral and spatial image distortions in a single cell caused by Bragg diffraction, which usually leads to a change in the spectra at individual points [13].

Registration of hyperspectral images was carried out in the Samara Regional Clinical Oncology Center. Informational consents of each subject were obtained. The study was approved by the ethical committee of the Samara State Medical University.

The result of registration with using the described setup of one sample is a hypercube, that consists of 151 optical images in .tiff format at wavelengths from 430 nm to 730 nm with a step of 2 nm. Each sample contained an area of neoplasm and healthy skin. Formation samples were taken from patients with European skin type. The final diagnosis was made for each examined sample on the basis of histological examination. Before further analysis, the hypercubes were reduced to 51 images in the range from 530 nm to 630 nm in 2 nm steps. The choice of this interval is conditioned by the best absorption of light by melanin and hemoglobin, as well as the high contrast of images, which facilitates image analysis. Using the software, individual grayscale images were obtained from the original hyperspectral images of the studied skin samples. The submission of images in this color scale allows to simplify the mechanism of interaction by reducing the input channels from three to one.

In total, 50 samples were used: 25 MM and 25 PN. This corresponds to 2550 optical images: 1275 MM and 1275 PN (see Table 1).

A CNN of the VGG architecture was used for image classification [14]. Unlike AlexNet, which focuses on smaller window sizes and steps of the first convolutional level, VGG addresses image depth [15]. The architecture of the neural network is shown in Fig. 2. The classifier consists of 13 layers. The number of trainable parameters increases as the layers deepen. The neural network classifier is divided into 5 blocks. The first 4 blocks are convolutional and each of them end with a maximum pooling layer.

Table 1 Composition of the DataSet.

No	Diagnosis	Number of samples	Number of images
1	Malignant melanoma (MM)	25	1275
2	Pigmented nevus (PN)	25	1275

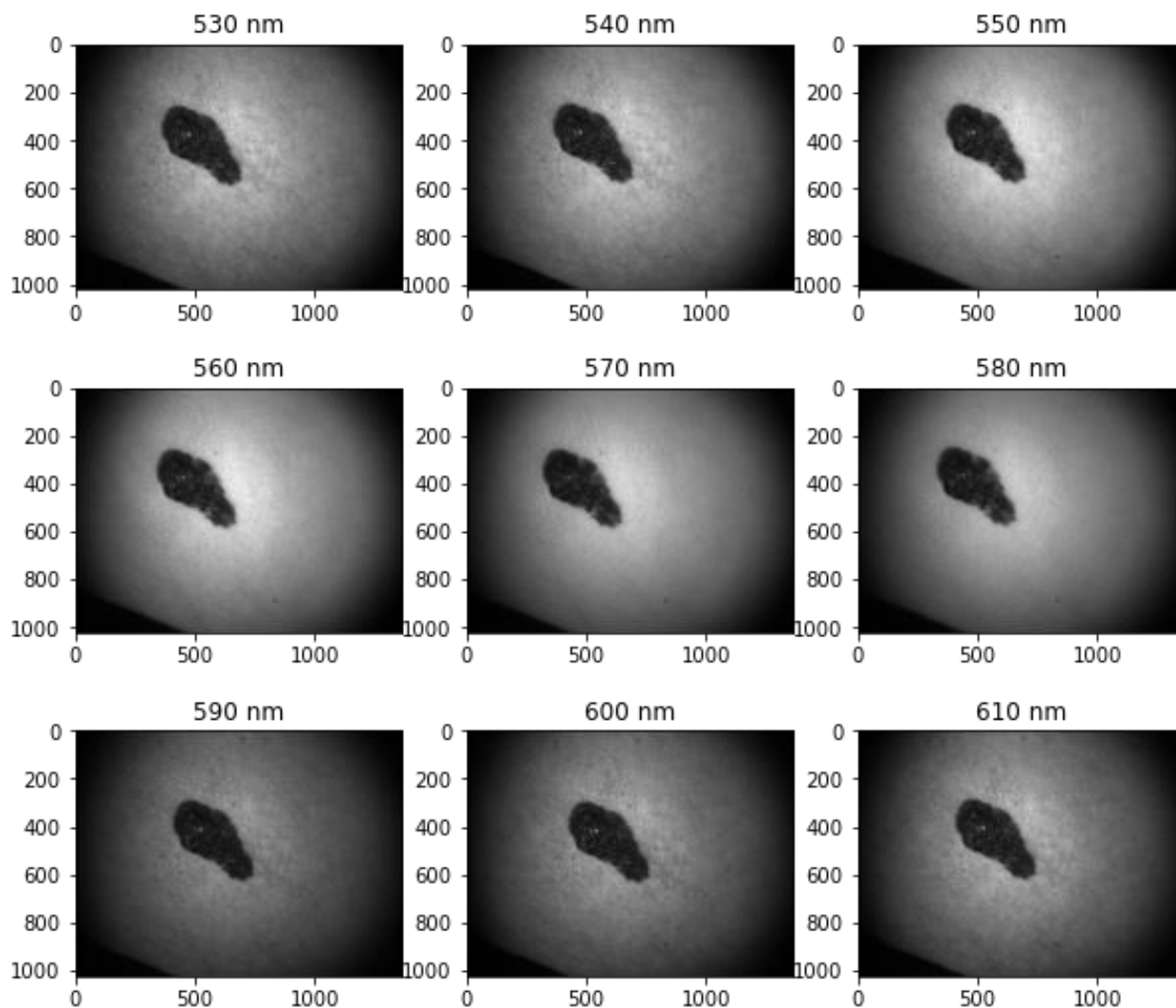


Fig. 1 Malignant melanoma at different wavelengths (the axes indicate pixels).

The input of the neural network is a two-dimensional RGB image, represented by a spectral slice and reduced to a size of 224×224 pixels due to additional scaling. The training and test samples were formed from images extracted from registered hypercubes in the specified range from 530 nm to 630 nm (see Table 1), in a ratio of 90% (2295 images) and 10% (255 images), respectively. The test sample set included only those images that did not participate in the training of the neural network classifier. In the training and test samples, a uniform distribution of samples across classes was made. This is caused by the need to obtain a more accurate classification at the stage of testing the operation of the neural network. Our classifier is trained using the backpropagation method. All layers of the network are fine-tuned using the same global learning network.

The neural network architecture also provides a feedback trigger that aborts training if the accuracy does not increase within three training epochs. This was provided to avoid retraining the model at the training stage.

3 Results and Discussion

The neural network was trained and tested on a computer equipped with an Intel(R) Core(TM) i7-10700KF 3.80 GHz processor, an NVIDIA GeForce RTX 3080 graphics card, and 32 GB of random access memory (RAM). The training time of the classifier was about 75 s (~5 s/epoch).

In the study, various variations of the last layer neuron deletion function were used, which consists in assigning zero to randomly selected features at the training stage. Thinning was added to the network through the Dropout layer, which you can see in Fig. 2. We used different thinning ratios of 10%, 15%, 20%, 25%, 30%, 40%, 50% of the features in the training phase. This was done to reduce the conspiracy of the model with the original data, thereby weakening the effect of overfitting. We found that increasing the removal percentage by more than 15% led to a decrease in the accuracy of the model. When thinning out half of the weights, the accuracy of the neural network classifier decreased to 76%. The highest accuracy, equal to 95%,

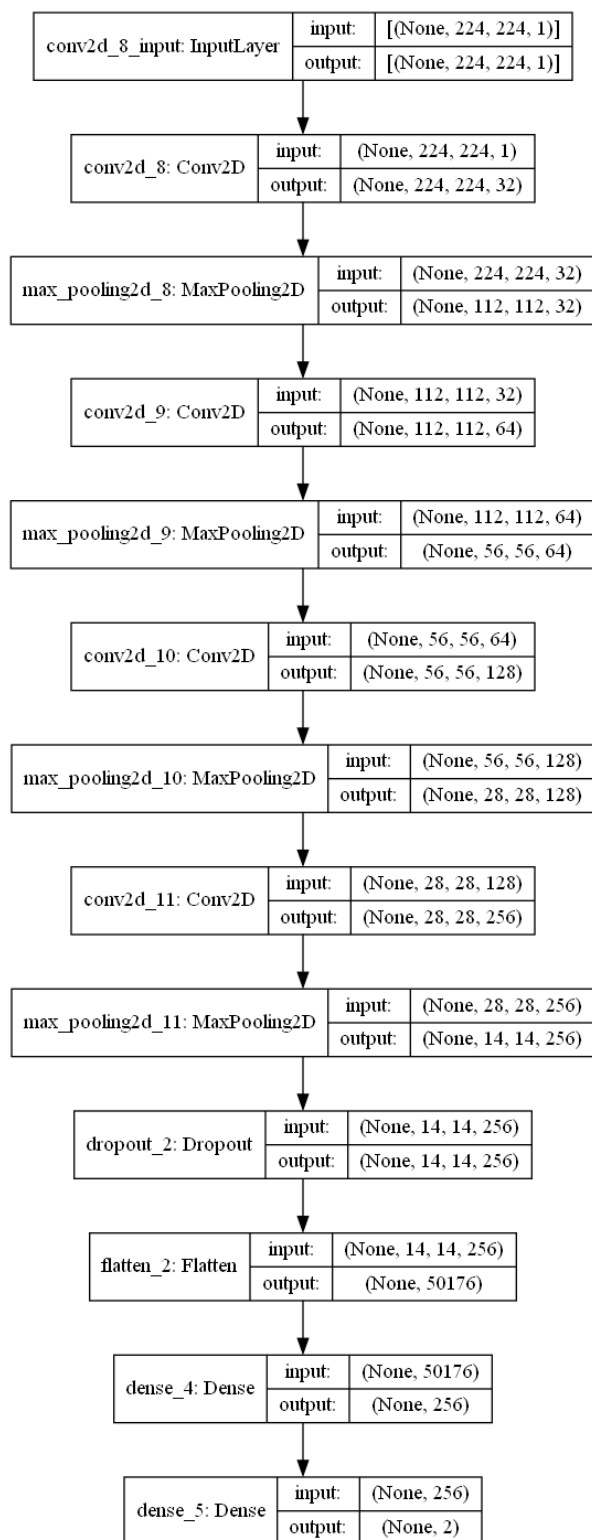


Fig. 2 The architecture of the used convolutional neural network.

was presented by the neural network when 15% of the features were removed. The model also presented the smallest loss (0.09) with the removal of 15% of features. To obtain accuracy and loss metrics, the values obtained during the validation test of the neural network were used. Accuracy metric is the ratio of the number of

correctly classified samples to their total number on the validation test and loss metric represents how well our model performs by comparing what the model predicts with the actual value.

The metrics obtained at the training stage of 15 epochs are presented in the Figs. 3 and 4. Then, class activation maps (CAM) were obtained on the example of a hyperspectral image of a pigmented nevus (see Figs. 5 and 6). This map of intermediate activations helps to debug the decision-making process in the neural network, especially in the classification error. It also helps to determine the location and specificity of objects in the image and draw a conclusion about the correctness of the neural network classifier. As it can be seen in Fig. 5, the neural network classifier focuses directly on the nevus itself, which indicates the correctness of the problem classification algorithms.

The obtained image classification accuracy of 95% on our test dataset allows us to say that the selected regions of the spectrum are informative enough to identify skin neoplasms.

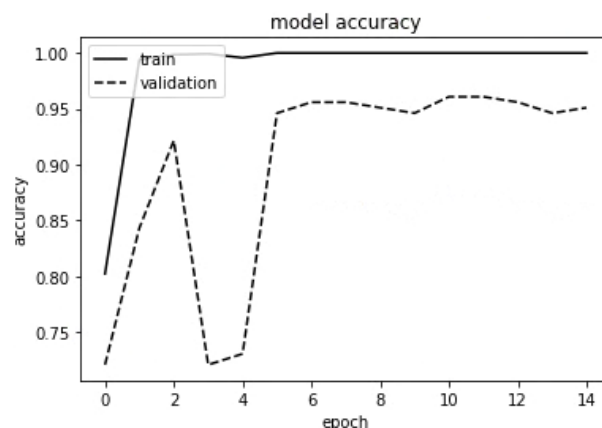


Fig. 3 Accuracy of the classification model with thinning out 15% of features: the solid line is the training set, the dotted line is the test set.

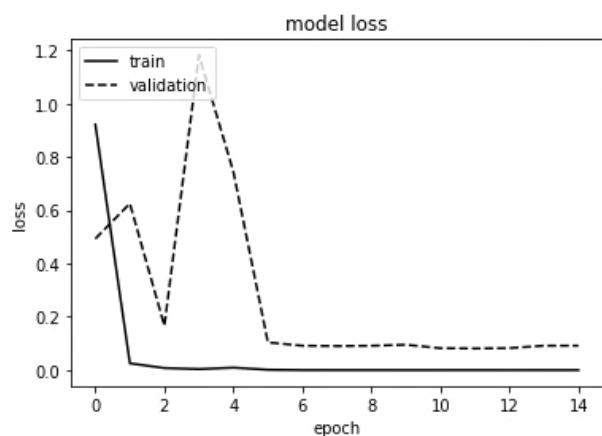


Fig. 4 Losses of the classification model with thinning out 15% of the features: the solid line is the training set, the dotted line is the test set.

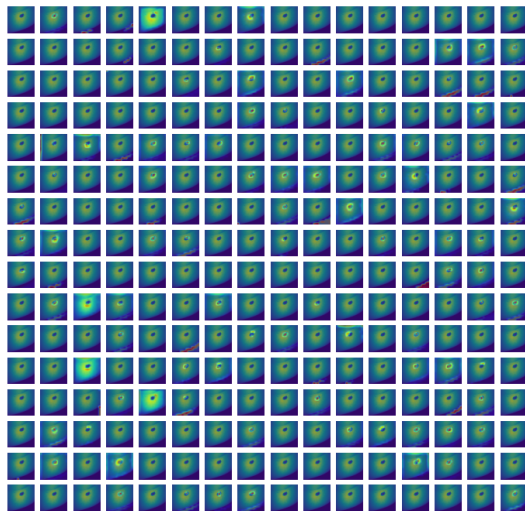


Fig. 5 Layer activations mapmax_pooling2d_11.



Fig. 6 Hyperspectral image of a pigmented nevus at a wavelength of 564 nm.

The obtained high accuracy of the binary classification of skin neoplasms is not only comparable with the results of the use of Raman scattering, but also exceeds the efficiency of fluorescence and dermoscopic examination [3]. An analysis of spectral indices calculated from hyperspectral images and characterizing differences in the relative content of skin chromophores for MM, basal cell carcinoma, and PN presented in Ref. [16] made it possible to obtain an accuracy of no higher than 78%.

Taking into account the small size of the training and test samples, the achieved accuracy values can both

increase and decrease as they increase. However, the obtained results demonstrate the possibility of using neural networks for the analysis of hyperspectral images in order to detect MM.

4 Conclusion

In this work, a binary classification of hyperspectral images of 50 samples of skin pathologies in the visible region was carried out using a neural network of the VGG architecture. The spectral ranges of 530–630 nm selected for differential image analysis showed high information content, which confirms the possibility of reducing the recorded spectral data and, accordingly, increases the speed of computational processes. The classification accuracy for MM and PN on test dataset was around 95%. The use of a hyperspectral image classification algorithm based on neural networks has improved the accuracy of skin pathology separation compared to other classification methods [7, 10, 11, 16].

Further development of technology is possible in several directions. The first is to conduct mass studies both with skin samples and with samples of other areas of human tissue to establish and improve the accuracy of diagnosing oncological diseases, as well as to compare the proposed method for classifying hyperspectral images with data on the accuracy of diagnosing other optical methods.

The second is the development of technology and the creation of an autonomous algorithm for the control of oncological diseases, which would allow monitoring the presence and condition of various unfriendly neoplasms.

The third is the optimization of the neural network architecture, the increase in the training sample and the modernization of hardware for registering hyperspectral images.

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Disclosures

The authors declare no conflict of interest.

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Monitoring Methods for Biocontrol of Robotic Wheelchairs

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Abstract. The challenges that arise in the process of developing robotic means of locomotion controlled by people with disabilities are examined in this paper. In addition, the methods of managing modern wheelchairs are analyzed. In order to prevent the occurrence of critical situations in the process of persons with disabilities movement, the ways of monitoring their condition are examined in the following work. Moreover, a comprehensive approach that increases the reliability of biocontrol of robotic wheelchairs has been proposed. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: neurointerface; eye-tracking; biocontrol; robotic wheelchair; condition monitoring; disabled people.

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1 Introduction

In the modern world, it is urgent to improve the quality of life and socialization of disabled people by enhancing the technical means that enable their mobility. This is considered to be especially significant for people with disorders of the musculoskeletal system [1] with the aim of increasing the safety of their movement in robotic wheelchairs with advanced means of control.

The primary challenges posed by robotic vehicles driven by people with disabilities are related to the poor quality of the control systems. As well as a high probability of stressful situations, which can cause critical biophysiological states that in turn reduce the effectiveness of management even more.

Modern methods of wheelchair control involve the use of a human-machine interface based on the perception of tactile, verbal, gesture, or visual commands. Recently, a new direction in this field has become widely spread. This approach is called a neurointerface (or brain-computer interface), based on the application of biocontrol methods that directly operate the signals of human brain activity it has advantages over other types of human-machine interfaces which are suitable for people with disabilities [2].

However, the usage of tactile, speech, and gesture methods of controlling a robotic wheelchair is not available in such cases where people lack the necessary motor functions. Therefore, the most promising method

to opt for is the employment of eye-tracking and neurointerface.

To prevent critical situations during wheelchair mobility, it is necessary to ensure high reliability of recognition of the robotic wheelchair control signals, which cannot be provided by each of the methods under consideration in isolation. In addition, regular monitoring of persons with locomotor disabilities during movement is necessary.

It is also important to use an individual approach to set up and test the control system, which can be achieved by applying operator and system training in virtual reality by simulating stressful driving situations.

The paper proposes a comprehensive approach based on a combination of virtual reality learning and control methods based on eye-tracking and neural interface. This approach will improve the efficiency and reliability of biocontrol of robotic wheelchairs.

2 Models and Methods

A number of current biocontrolled wheelchair designs are based on an eye monitor interface, the use of such an interface reduces the viewing angle and complicates wheelchair control. Eye gesture detection is effective in the design of a robotic wheelchair because it avoids the need for a monitor interface [3].

The generalized algorithm of the eye-tracking method includes the following steps:

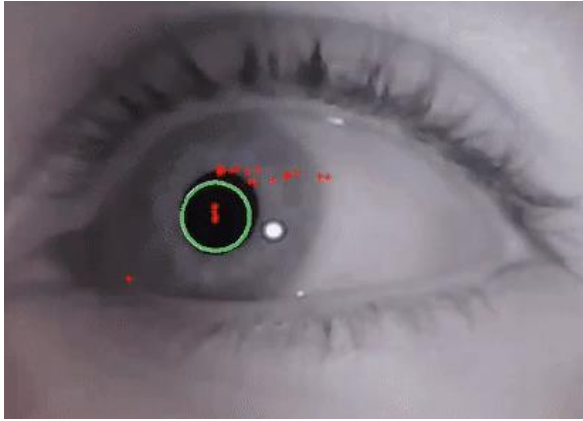


Fig. 1 The eye-tracking calibration process.

- eye-tracker calibration using standard hardware and software, video camera scanning of the reflected near-infrared signal received by the subject's pupil;
- software filtering and correction of deviations to determine the current coordinates of the gaze position, which are taken into account when controlling the robotic wheelchair.

The disadvantage of using the eye-tracking method is that the parameters transmitted to the control mechanisms need to be constantly updated, assessing the accuracy of the eye movement data of a particular subject. At the same time, the accuracy of the eye tracker's real-time measurements of eye coordinates is significantly affected by various types of interference and stochastic conditions.

Let us consider a generalized eye-tracking algorithm.

1. The eye-tracker calibration process is performed using standard hardware and software.
2. Eye-tracker emits light in the near infrared range.
3. The eye-tracker light is reflected in the subject's eyes.
4. The reflections are recorded by the eye-tracker reading camera (Fig. 1).
5. The coordinates of the current position of the gaze after filtering are determined.

The gaze position coordinates (X and Y values in pixels) must be converted to frontal gaze angles and gaze offset and tilt angles for further processing. The unprocessed left and right eye gaze coordinates (X_{left} , Y_{left} and X_{right} , Y_{right}) obtained from the eye tracker are used to estimate gaze angles and gaze offset [4]:

$$GazeX = \text{mean}\left(\frac{X_{left} + X_{right}}{2}\right), GazeY = \text{mean}\left(\frac{Y_{left} + Y_{right}}{2}\right).$$

The display distance (RD) is the distance on the screen between a coordinate point and a specific viewpoint with coordinates (G_x , G_y). For example, if the tracker is fixed directly under the screen, and the source of the coordinates is the center of the screen, we get the following expression:

$$RD(mm) = p\left(\sqrt{(G_x)^2 + (G_y)^2}\right),$$

where p is the pixel pitch of the display in units of mm/pixel.

After obtaining the coordinates, further data processing in the eye tracker channel consists of the following procedures: detection of fixations and saccades. It should be noted that “fixations” and “saccades” detected by the software are the result of a special neural network algorithm of data processing with certain training network settings to increase the detection efficiency.

One biofeedback channel is not enough to provide the necessary reliability of recognition of commands of the person under study, therefore, to increase the reliability and safety of the biofeedback interface, an additional parallel channel should be chosen.

Currently, the brain-computer interface is being actively developed all over the world, its implementation is technically quite complicated and requires a long personal sets.

Let us consider using brain-computer interface as an additional biofeedback channel.

Brain-computer interfaces (BCIs) provide a direct pathway from the human brain to the computer using techniques used to record and decode brain output. The first BCI systems were used primarily for stroke rehabilitation. More recently, BCIs have been widely used to improve the quality of life of patients with disabilities while operating assistive devices such as electric wheelchairs and prosthetics. Recently, BCIs have been widely used not only for people with disabilities, but also for people without disabilities. These BCIs are mostly noninvasive systems with an electroencephalogram (EEG) monitoring function that can be integrated into portable devices.

In general, BCIs contain five main processing steps: data acquisition, preprocessing, information feature extraction, classification, and feedback. EEG signals have a low signal-to-noise ratio and tend to be nonstationary over time. Expert knowledge, prior knowledge, and judgment about the subject domain are traditional bases for developing methodology and training classifiers that apply only to specific datasets. Such a strategy is not easily scalable to any other experiment or dataset.

The physiological basis of the correlation between brain activity and motor activity is that body movement can generate mu (8–12 Hz) and beta (16–26 Hz) rhythms with event (de-)synchronization (ERS/ERD) in motor-sensory brain regions [4]. Some studies of motor activity-based devices (wheelchairs, prostheses, robots) have medical applications and provide further development of complementary technologies for humans. Common feature extraction algorithms for classification of motor activity from EEG are based on detection of spatial patterns (SPs).

The idea of Common Spatial Pattern (CSP) is to find a set of spatial filters that optimally distinguish several

classes of EEG recordings. The filter-bank CSP (FBCSP) algorithm, which uses a feature selection operator, allows selecting the most optimal spatial filter for feature extraction. The advantages of this method are its simplicity and accuracy. Other CSP-based methods similarly extract valuable components of EEG signals after special software analysis.

Unfortunately, EEG characteristics change significantly over time and vary from person to person. Therefore, it is necessary to create more reliable and more generalized algorithms for feature extraction, especially for biocontrol applications.

Deep learning has made great strides in computer vision, natural language processing, and speech recognition. Current trends in these advances seem to suggest that neural computing units, such as convolutional layers in convolutional neural networks (CNNs), can extract implicit features from signals to improve system performance.

Deep learning development is also a promising one, particularly in EEG feature extraction. However, the application of Deep learning in EEG-based interface control system (ICS) has two problems: first, the low signal-to-noise ratio (SNR) and time-dependent covariates of the EEG signal make feature extraction difficult, and second, the insufficient data set and individually different EEG signals among subjects result in low artificial neural networks learning efficiency.

A multi-channel method of controlling a mobile robotic wheelchair is known, which implements an extended brain-computer interface, with the control system built on the parallel operation of independent channels (neurointerface, speech and gesture control). However, in complex situations, this approach leads to a “conflict of interest” due to the inability to simultaneously execute opposite commands from multiple channels. To resolve such conflicts, methods of coordinated control and decomposition based on quality assessment of control channels are employed [5].

3 Results

This paper proposes an integrated approach based on the coordinated application of the two most promising methods of wheelchair biocontrol, namely eye-tracking and neural interface, with the commands either confirming each other, or using only the channel that the control data processing system gives priority to at a particular point in time. Thus, even in the case of conflicting user commands, the proposed approach ensures high reliability of the wheelchair control.

Experimental studies of EEG signals under normal and stress conditions using NVX24 equipment of MKS LLC (Moscow) are being actively carried out, an example is shown in Fig. 2.

Thirty people (including people with disabilities) aged 21 ± 3 years participated in this study for 3 months. Participants performed a series of special tasks over several sessions, yielding a total of 760 trials. All

recording sessions were performed in our laboratory on MCS NVX24 digital DC EEG 2.02 equipment.



Fig. 2 Example of experimental EEG signal extraction using dry gel from MKS.

EEG signals dataset has examples per trial total of 2870 trials. Augmentation techniques were used to augment the data collected. CNNs can successfully classify spectrograms. In this study, it is appropriate to analyze the data using the classical medical features that are standard in EEG spectral analysis. Thus, we introduce a parallel CNN to classify integrated eye-tracking and EEG results.

The architecture of the CNN is presented in Fig. 3. Detailed architecture of the proposed network is presented in Table 1. In the Table 1, T is the number of time points, C is the number of channels, F_S is the spatial filter, F_T is the time filter, D is the ratio of F_T to F_S , N_C is the number of classes. In the proposed method, we introduce a parallel CNN with 2 inputs – for data from the eye tracker channels and 4 inputs – the signal amplitude of a certain EEG frequency.

In the proposed architecture, the output feature maps are fed into a spatial convolutional module, with the kernel length of the temporal filter F_T set to 32 and 16. Dropout layer follows the pooling layer to avoid overfitting, where dropout rate $p = 0.5$.

In the proposed study, we use a network learning algorithm similar to the CNN learning algorithm. To test the feasibility and effectiveness of our proposed method, we conduct a series of motor imagery (MI) classification experiments. The results on the datasets prove that the CNN performance has a predictive value of 76.32.

As part of this integrated approach, an original algorithm and methodology for setting parameters of the neurointerface and testing the wheelchair control system based on the virtual reality glasses and simulation of various traffic situations are being developed (Fig. 4).

The results of the experimental evaluation of the EEG parameters in different stress situations in the group of persons with disabilities under study show that the highest values of the average signal power deviation ratio values in the “stress/peace” categories are found in the delta and beta rhythms of the EEG.

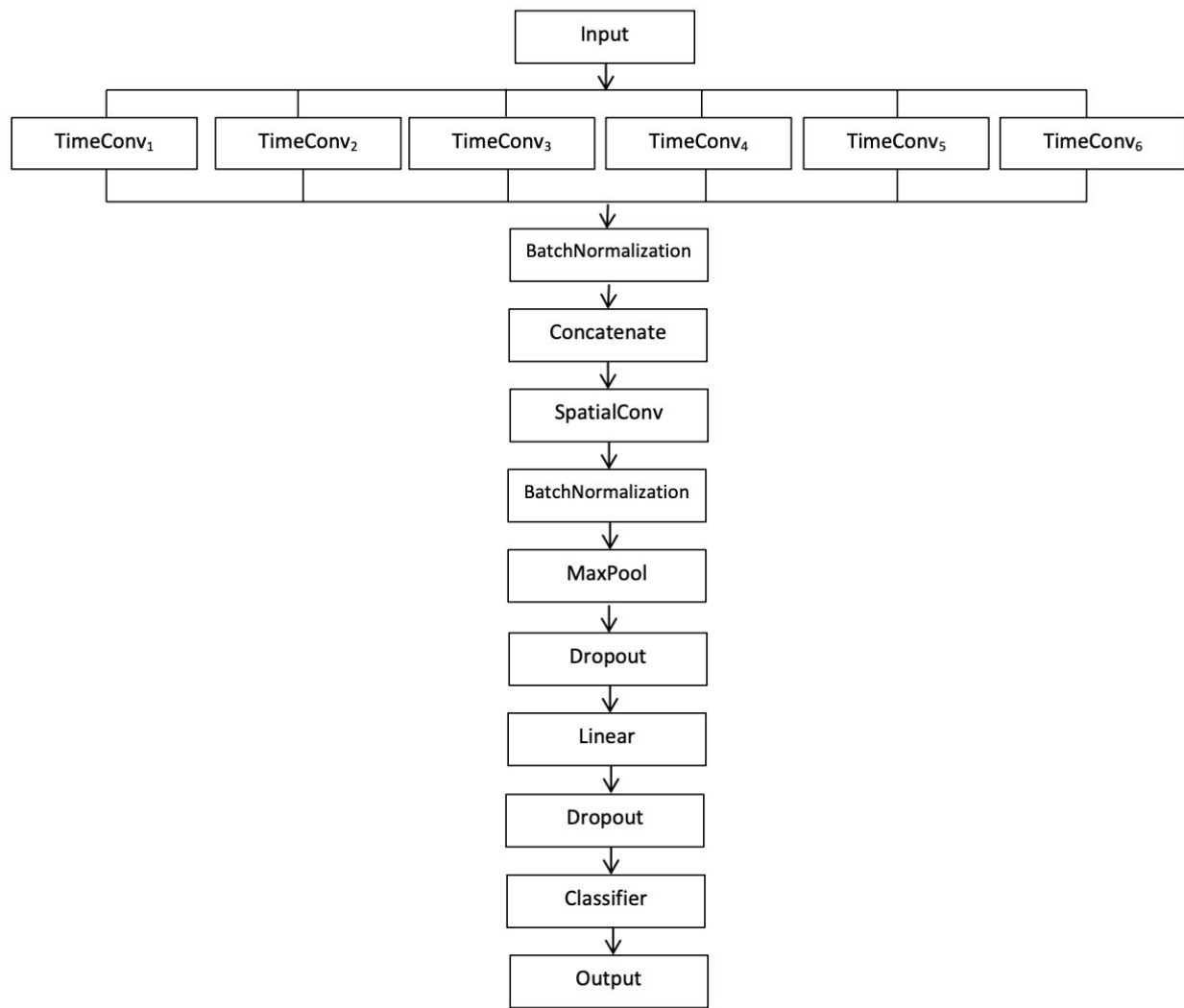


Fig. 3 The architecture of CNN.

Table 1 Detailed architecture of the proposed network.

Layer	Filters	Kernel	Stride	Parameters	Output	Activation	Padding
Input					(C, T)		
Reshape					$(1, T, C)$		
TimeConv ₁	F_T	$(16, 1)$	$(1, 1)$	$16 * F_T$	(F_T, T, C)	ReLu	Same
TimeConv ₂	F_T	$(16, 1)$	$(1, 1)$	$16 * F_T$	(F_T, T, C)	ReLu	Same
TimeConv ₃	F_T	$(32, 1)$	$(1, 1)$	$32 * F_T$	(F_T, T, C)	ReLu	Same
TimeConv ₄	F_T	$(32, 1)$	$(1, 1)$	$32 * F_T$	(F_T, T, C)	ReLu	Same
TimeConv ₅	F_T	$(32, 1)$	$(1, 1)$	$32 * F_T$	(F_T, T, C)	ReLu	Same
TimeConv ₆	F_T	$(32, 1)$	$(1, 1)$	$32 * F_T$	(F_T, T, C)	ReLu	Same
BatchNorm				$2 * F_T$	$(4 * F_T, T, C)$		
Concatenate					$(4 * F_T, T, C)$		
SpatialConv	F_S	$(1, C)$	$(1, 1)$	$C * 4 * F_T * F_S$	$(F_S, T, 1)$	ReLu	Valid
BatchNorm				$2 * F_S$	$(F_S, T, 1)$		
MaximumPool		$(75, 1)$	$(15, 1)$		$(F_S, T//15, 1)$		Valid
Dropout					$(F_S, T//15, 1)$		
Linear					$(F_S, T, 1)$	Square	
Dropout					$(F_S, T//15, 1)$		
Classifier	N_C	$(T//15, 1)$	$(1, 1)$	$F_S * (T//15) * N_C$	N_C	Linear	Valid



Fig. 4 An example of a virtual reality scene for teaching people with locomotor disabilities.



Fig. 5 Mason A4100 smartwatch for patient monitoring.

Thus, for biocontrol in general, and for individual adjustment and testing of neurointerface operation it is reasonable to choose these very EEG channels [6]. In order to prevent critical situations in the process of disable people's movement, it is essential to ensure high reliability of recognition of robotic wheelchair control signals, which cannot be provided by each of the methods considered in isolation. In addition, permanent monitoring of persons with locomotor disabilities during their mobility is required.

It is also of great significance to employ an individualized approach to setting up and testing the control system, which can be ensured by applying operator and system training in virtual reality through simulation of stressful driving situations.

The paper proposes an integrated approach that is based on the combination of virtual reality training and control methods powered by eye-tracking and neuro-interface, allowing improving both the efficiency and reliability of biocontrol of robotic wheelchairs. The occurrence of critical health impairments among disabled people during their movement in mobile wheelchairs can lead to emergency situations, in order to prevent which the ways of monitoring their condition have been analyzed and the following equipment has been selected.

The Mason A4100 smart watch [7, 8] provides blood pressure monitoring, pulse measurement and control,

user identification by ECG, breath and stress monitoring, location, and fall tracking (Fig. 5).

The H8S-PRO complex (Fig. 6) is designed for a wide range of mobile studies with tracking of neurodynamics and visual behavior and is produced by Neurobotics LLC (Moscow) [9].

In the future, to monitor critical states associated with cramps and muscle tremors, it is planned to use Neurosens inertial sensors produced by Neurosoft, which transmit three-dimensional coordinates of the sensor position on the body and the process of movement to a notebook computer (Fig. 7). In addition, this sensor has a modification in which the electromyographic signal is withdrawn [10].

The practical implementation of such systems is widely discussed around the world. The analysis of achievements in this area has shown the promise of developing an individual user interface, which can be used both for private use and to match the parameters of a particular person with serial devices of universal type, with multilevel customization based on additive manufacturing technologies [8].



Fig. 6 Complex for mobile tracking H8S-PRO.



Fig. 7 3D motion sensor.

4 Conclusion

In this paper, the most applicable ways of monitoring health conditions during the process of movement of disabled people to prevent the occurrence of critical situations are selected. In addition, it is proposed to employ an integrated approach based on a combination of eye-tracking and neurointerface methods. This allows increasing the reliability of mobile wheelchair

biocontrol, during which the biophysical parameters are constantly monitored and analyzed. What is more, the inhibition mechanism is activated when critical states are detected.

Disclosures

The authors declare no conflict of interest.

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Electronic Contact Lenses as a 3P Medicine Tool: the Electrode Lead System Modeling

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Abstract. Electronic contact lenses (ECL) are a high-tech solution for diagnosing, including early, both ophthalmic and systemic diseases. The authors of the article propose the implementation of 3P medicine approaches by integrating electrodes and circuits into the ECL to realize bioelectrical impedance spectroscopy (BIS) of the anterior eye. This helps to analyze the blood circulation parameters of this part of the eye. Electrode lead system modeling integrated into the contact lens was carried out by using the finite element method. It was obtained for the correct operation, the presence of an electrically insulating layer between the biological tissues of the eye and the main material of the contact lens is necessary. The electrode diameter is 0.8 mm, and the optimal distance between the current electrodes should be 6 mm, between the potential – 4 mm. Assessment of the power level required for BIS shows the possibility of technical implementation of this methodology from an energy point of view. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: electronic contact lenses; ECL; biomarker; 3P medicine; bioimpedance; electrodes; lead system.

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1 Introduction

The modern level of science and technology makes it possible to manufacture miniature devices for solving a wide range of medical problems. For example, electronic contact lens (ECL) is one of the high-tech solutions that significantly expand diagnostic and therapeutic possibilities in ophthalmology. The main advantage of ECL is the possibility of patient long-term wear. This allows us to analyze changes in a particular parameter over a long period, and not at separate moments when the patient is in a medical institution. The work [1] describes ECLs used for daily monitoring of intraocular pressure (IOP). Other developments show that it is possible to control systemic parameters, for example, the level of glucose in the blood that the corresponding level of glucose in the tear. This relationship was proved only in 2020 [2].

Many laboratories around the world develop miniature devices underway aimed at integrating classical or innovative sensors and circuits, including implementing wireless technologies for transmitting energy and information, into contact lenses

2 3P-Medicine, Tear Biomarkers, and Functional Indicators of the Anterior Part of the Eye

In the example of glucose control, it can be seen that the use of ECL allows the realization of personalized treatment. This means that you can find the required amount of medication for a particular person in a particular situation by focusing on the current concentration of glucose in his tear. Analysis of the dynamics of glucose levels makes it possible to determine objective criteria for predictive diagnosis, i.e.

predicting the nature of the development of the disease in a particular patient and choosing preventive treatment tactics on this basis. Thus, the 3P principle is realized: predictive, personalized, preventive medicine.

As known these principles began in the early 2000s, but they only became widespread a decade later, when it became obvious that the reactive approach in medicine was not effective [3].

As a result, the European Association for Predictive, Preventive, and Personalized Medicine (EPMA) was formed in Europe in 2008 [4]. The above principles are enshrined in law in the Russian Federation by the Order of the Ministry of Health of the Russian Federation No. 186 dated April 24, 2018 “On Approval of the Concept of Predictive, Preventive and Personalized Medicine” and included by Order of the Government of the Russian Federation dated December 28, 2012 No. 2580-r in the Strategy for the Development of Medical Science in the Russian Federation for a period until 2025. It is worth noting that in some publications you can find such terms as 4P- and 5P-medicine [5, 6]. The fourth P means participation, i.e. the patient must be involved in the process of diagnosis and treatment. The fifth P is psycho-cognitiveness. That means that the provision of medical services should take place taking into account the psychophysical state of the patient during diagnostic studies, and therapeutic and surgical manipulations, and, accordingly, the involvement (fourth P) of the patient will be more effective.

Human tears as one of the biological fluids contain a wide set of biomarkers. It is known that there are certain correlations between the concentrations of various components in tears and blood. Some examples of these are given in Table 1 [7].

Table 1 Concentrations of different components in tears and blood [7].

Component	Tear concentration	Blood concentration
Na ⁺	120–165 mM	130–145 mM
K ⁺	20–42 mM	3.5–5 mM
Glucose	0.1–0.6 mM	4.0–6.0 mM
Urea	3.0–6.0 mM	3.3–6.5 mM
Lactate	2–5 mM	0.5–0.8 mM
Pyruvate	0.05–0.35 mM	0.1–0.2 mM
Ascorbate	0.008–0.04 mM	0.04–0.06 mM
Total protein	~ 7 g/L	~ 70 g/L

It is worth noting that the measurement of the concentrations of certain biomarkers in a tear is associated with certain difficulties associated with the fact that the tear fence should not be unsimulated. So, as

noted above, the direct dependence of the concentration of glucose in a tear on the concentration of glucose in the blood was proved only in 2020 [2]. Previously, this could not be proved for few years, because, as it was found later, a small amount of blood fell into samples of tear fluid.

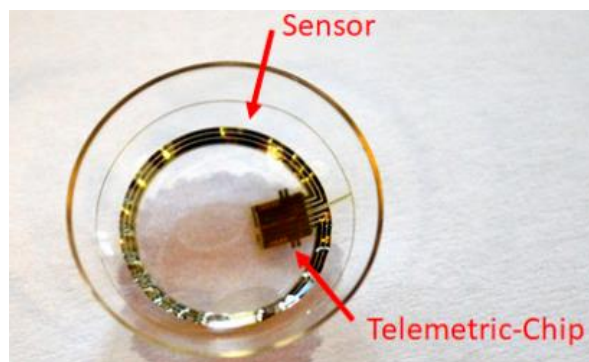
Contact lenses can help to solve the issue of the correct fence of tear fluid, as well as ensure the possibility of prolonged and continuous monitoring. Ordinary contact lenses are already widespread, can be worn for a long time, and do not cause significant discomfort to the patient. The modern level of microelectronics and technology allows to integration electronic circuits and sensors directly into contact lenses, thereby allowing to obtain high-tech minimally invasive diagnostic devices.

3 Electronic Contact Lenses

Each of these indicators, individually or in combination, can be used as a biomarker that is informative in the diagnosis and monitoring of some diseases. This correlation makes it possible to use tear fluid indicators as diagnostic and prognostic criteria not only for ophthalmic but also for systemic diseases, for example, in Alzheimer’s, Parkinson’s, thyroid gland, in some types of oncology, etc. [8]. In addition to chemicals in the tear, biomarkers can be physiological indicators, such as the IOP, the determination of which is necessary for the diagnosis and control of glaucoma. In modern ophthalmic practice, the analysis of lacrimal fluid is not very widespread, because it is to the complexity of accurate and unstimulated sampling of this biological fluid. This procedure can be made only by medical staff in a medical institution. This is especially related to measuring physiological parameters, for example, IOP.

As is said above electronic contact lenses can help solve the problem of long-term monitoring of biomarkers because the patient can wear them for a day or more. The modern level of microelectronics and technologies makes it possible to integrate biosensors and digital electronics with information and energy transmission channels into smart contact lenses.

The ECLs are the reality today because they have already been used in clinical practice since 2016 when the TriggerFish product (Switzerland) received approval from the US Food and Drug Administration (FDA) for clinical use in the US [9]. Fig. 1 shows a general view of such a product and its placement on the eye for measuring IOP during the day [10]. In the literature, one can find the results of laboratory studies aimed at integrating sensors of various types into ECLs [8]. The ECL measures the IOP during the day and is used to evaluate high-quality the minimum invasive surgery for the treatment of glaucoma or cataracts has been performed. It is known that the IOP has daily fluctuations. IOP is recorded before and after surgery by using ECL. In case of circus rhythms are not significantly disturbed, this means that the surgical operation was performed qualitatively and the patient has a favorable forecast for recovery after surgery.



(a)



(b)

Fig. 1 TriggerFish electronic contact lenses for measuring intraocular pressure [10], (a) ECL photo, (b) ECL on the eye.

Bauman Moscow State Technical University in cooperation with Helmholtz National Medical Research Center of Eye Diseases is developing ECL, in which it is planned to integrate a tetrapolar electrode lead system for the implementation of bioelectrical impedance spectroscopy (BIS) of the hemodynamics of the anterior eye. This technique appeared in the 60s, one should use double anesthetics and put on a suction cup on the open eye. Development is based on the experience of the above team on the of a transpalpebral research methodology development, i.e. through a closed eyelid, which makes it possible to refuse anesthesia [11]. The BIS makes it possible to study the parameters of blood circulation in the anterior eye, which are informative biomarkers of many ophthalmic diseases, for example, myopia, especially in the early stages of development, including in children. Work results on the development of a transpalpebral methodology were introduced into the work of the Helmholtz National Medical Research Center of Eye Diseases.

The logical development of the above technology is the integration of the electrode system in ECL. This makes it possible to implement the approaches of 3P medicine and control the dynamics of pulse blood filling of the anterior eye, which is impossible when using the transpalpebral technique or any other that is carried out

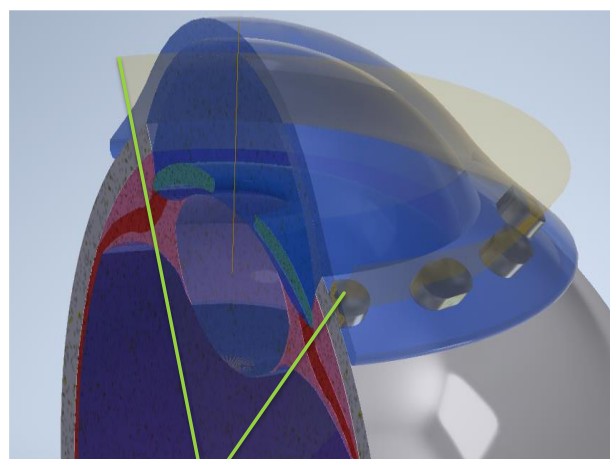
exclusively in a medical institution. Among the existing ECL developments that analyze various biomarkers of the eyes, most developments are aimed at the biochemical indicators of tears. A separate ECL class is aimed at analyzing intraocular pressure, or rather the dynamics of its physiological parameter. This is because the sensors are installed not inside the eye, but from the outside, and evaluation of intraocular pressure is based on some mathematical models according to indirect signs. The literary review did not reveal developments aimed at the implementation of bioelectrical impedance spectroscopy in the ECL. In this regard, there is no information about geometric and constructive parameters of electrode lead system in the literature.

We have simulated the BIS by the finite element method when the electrode lead system is integrated into the ECL. We can use a three-dimensional model developed in the work [12], which is shown in Fig. 2. It is an anatomically reliable three-dimensional model of the eyeball with located electrodes and, considering the blood supply system of the eye.

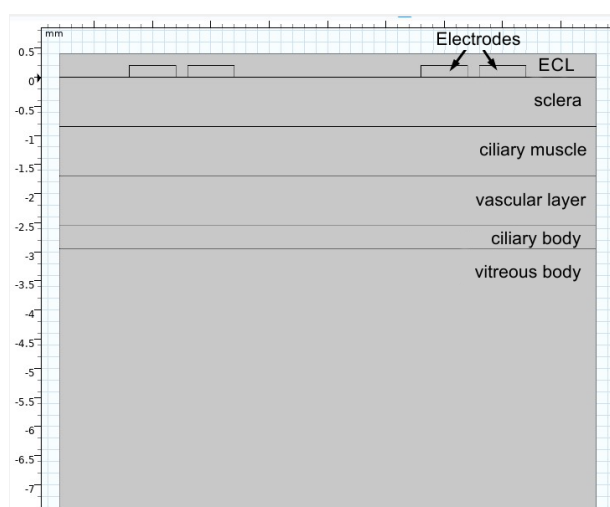
As a rule, the presence of blood circulation in the eyeball models is considered an artifact and neglect it. However, in this case, blood circulation is a central object of study and cannot be neglected. It is considered that the eyeball consists of about 40 different types of tissues, in the model geometry is 9 of the most pronounced tissues of the eye, one of which is the blood structures of the eye (shown in Fig. 2(a) in red color in the model). The geometry is based on an anatomical atlas having dimensional scales. The assumption is also made that the parameters of the blood supply layer are represented by blood parameters.

BIS is carried out at a frequency of 100 kHz and a current of 4 mA. It is possible to use a double-electrode or four-electrode research scheme. Because when using the four-electrode scheme, the resistance of the electrode-tissue does not fall into the measured value, such a measurement scheme is considered preferable. In such a study, two electrodes are used to skip high-frequency current through tissues and are located outside, and two other electrodes are located between them for measurement. Due to this, there are no significant restrictions on the placement of the electrodes, then the framework of this work investigates a 4-electrode connection scheme. All electrodes, as a rule, have the same geometry.

Since the object of the study is the most blood supply of the anterior eye, the electrodes for the ECL should be located as close to them as possible. During anatomy and on the three-dimensional model it is clear that the most optimal location will be the electrode's location near the eye limb. Because of this, the model of symmetry can be distinguished from the model, which looks in the form of a cone (shown in Fig. 2(a) in the form of an orange surface and green lines in a cross-sectional plane). This surface passes through all the electrodes. The angle between the forming and the axis of the cone is selected so that the surface passes through the widest part of the circulatory layer and the geometric center of the eyeball.



(a)



(b)

Fig. 1 Models of the eyeball. (a) A three-dimensional model of the eyeball with installed electrodes, (b) layers in a two-dimensional model of the eye.

Within the framework of this work, based on the above surface, a flat model was formed, which includes five layers of the eyeball, electrodes, and directly the material of the contact lens, which is shown in Fig. 2(b).

Table 2 shows the specific electrical conductivity and relative dielectric permeability of individual eye tissues at a frequency of 100 kHz used in the model [13]. The current source is set on the right electrode, the left electrode was used as grounded. Because the ECL is installed on the open eye, the system is moistened with tear liquid, and the conjunctiva, presumably, does not have any electrical insulating properties, it is assumed that there is no resistance between electrode and tissue.

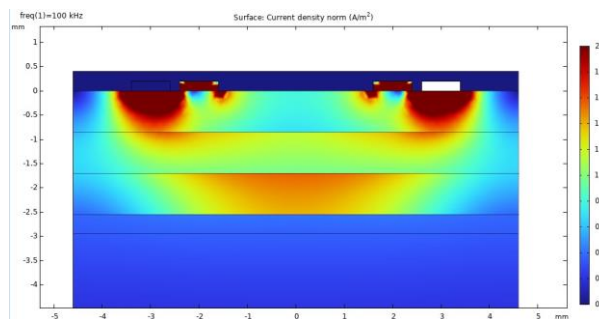
It was obtained that for correct BIS, it is necessary that an electrically insulating layer is necessary between the eye tissues and the material. Only the surface of the electrodes should be in contact with the surface of the sclera. Otherwise, if the electrical conductivity of the ECL material is comparable to the electrical conductivity of the eye tissue, then almost the entire current passes through the ECL, which makes the BIS study of the

vascular layer of the anterior eye extremely unfortunate to changes in the pulse blood supply.

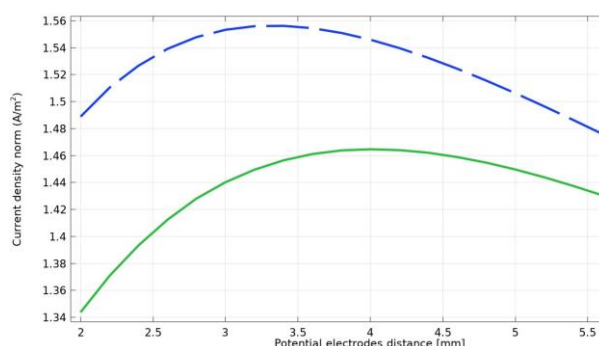
If we use an electrical insulating layer between the tissue and the ECL, the voltage on potential electrodes is 7.9 mV. Fig. 3(a) shows the resulting distribution of current density in the eye tissues. Pulse blood supply was modeled by a change in the conductivity of the vascular layer. The level of blood supply to the eye is about 1 ml per minute, and on average 0.018 ml of blood flows in the eye. That is why, the increment of the conduction of the vascular layer in one heart blow will be about 0.3%. The amendment to the model showed that the amplitude of the recorded pulse blood supply should be 5.6 μ V. This is comparable to the real voltage levels recorded in BIS studies of other areas of the body, and can be measured by ordinary electronics.

Table 2 Eye tissue properties used in the model (100 kHz) [13].

Eye tissue	Conductivity, S/m	Relative permittivity
Sclera	0.5	37.5
Ciliary body	0.40	66.1
Vascular layer	1.35	64.2
Ciliary muscle	0.84	58.8
Vitreous body	0.40	66.2



(a)



(b)

Fig. 3 Simulation results. (a) Current density distribution [A/m²] in the eye tissues of the anterior eye. (b) Current density in the vascular layer: the dotted line is the maximum, and the solid line is the average.

The voltage on the current electrodes was 15 mV, so the necessary power for the study is about 60 μ W. Compared to ECL, which is developed by other teams, this value is small, even considering that when implementing the methodology, it will be necessary to implement other electronic components from measuring cascades to the wireless transmission and energy transmission schemes [14]. This confirms the potential for the integration of electrodes for EI in electronic contact lenses.

The optimal electrodes location was studied during the simulation. Fig. 3(b) shows graphs of the current density as a function of the interelectrode distance. The distance between the potential electrodes was changed by step 0.1 mm, the center of current electrodes was located at a distance of 1 mm from the center of potential ones. The size of the electrodes was 0.8 mm in diameter. The results showed that the maximum current density at a single point of the vascular layer can be achieved with an interelectrode distance of 3.4 mm. However, it is not correct to use the maximum value of the current density in a separate layer for BIS; it is more correct to use the average value, which reflects the total amount of current passing through the vascular layer. In this case, the optimal location of potential electrodes is at a distance of 4 mm, and current electrodes are at a distance of 6 mm. This will give the greatest sensitivity to pulse fluctuations in the blood filling of the anterior eye at the depth of the ciliary body. Moreover, with such an arrangement of the electrodes, there is no such significant difference in current densities within the boundaries of one vascular layer, as in the presence of the maximum found value, i.e. with a wider arrangement of electrodes, the current density is distributed more evenly, which will contribute to the fact that the integral estimate of the density while in the desired layer will be more correct.

4 Conclusion

The ECL alloys wide opportunities allow you to realize modern concepts in the organization of healthcare – 3P-medicine. The main advantage of the ECL is the possibility of prolonged non-invasive monitoring of biomarkers of both ophthalmic and systemic indicators

of the body. Most of the existing works of large scientific groups are aimed at developing ECL for the analysis of biochemical markers contained in a tear. For this, innovative sensory systems are developed, but there are almost no sensors for the analysis of physiological indicators of the eye, except for systems for analyzing intraocular pressure. The electrode lead system in an electronic contact lens proposed in the work will implement the methodology of electroimpedance research. This will analyze the level of blood supply of the anterior eye. At the same time, no other method, widespread in ophthalmology, does not allow analyzing the level of blood supply to the front part of the eye. Based on the proposed two-dimensional model, modeling the distribution of currents in the fabrics of the front department of the eye was carried out during BIS in case the electrodes are located decently, i.e. on the sclera along the iris of the eye. Modeling made it possible to determine the optimal location of the electrodes in the electrode lead system, which is supposed to integrate into the ECL. It is 4 mm between potential electrodes and 6 mm between current ones with an electrode size of 0.8 mm. In this case, the presence of an electrically insulating layer between the tissues of the eye and the main material of the contact lens is necessary. In addition to the development of the diving system directly, it is necessary to develop schematic solutions for the implementation of the BIS methodology, power schemes, and information transmitting through wireless communication channels, while they should be integrated into contact lenses and have the properties of bioadexcence and biocompatibility. This is material for subsequent research and development.

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Disclosures

The authors declare no conflict of interest.

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Possibilities of MRI Texture Analysis of Brain Images in Differential Diagnosis of Primary Extra-Axial Tumors

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Abstract. The possibility of using textural features for the differential diagnosis of primary extra-axial tumors on the basis of two-dimensional digital magnetic resonance (MR) images is being investigated. Histogram and correlation characteristics, as well as features based on run lengths, were considered as information features. The selection of effective features is based on the criterion of discriminant analysis. The applicability of the proposed approach to solving the problem of differential diagnosis between different types of extra-axial tumors is being studied experimentally on a set of 66 patients. The analysis of standard MRI data based on radiomics showed a better differential diagnosis result 33.3% superior to visual assessment. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: radiomics; texture analysis; diagnostics; feature selection; discriminant analysis; MRI.

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1 Introduction

Meningiomas and neurinomas are the most common intracranial primary tumors [1]. This is a heterogeneous group of tumors, including both benign and malignant tumors. The approach to treatment largely depends on the type of tumor, so the problem of differential diagnosis of primary extra-axial tumors (PExT) based on magnetic resonance imaging (MRI) images does not lose its relevance [2–4].

Currently, the interpretation of changes detected on medical images is based on visual analysis of a limited number of qualitative and quantitative criteria (localization, number, shape, size, structure, etc.). However, in addition to this small number of radiological features, the imaging data is rich in quantitative parameters (shape descriptors, histogram and texture characteristics) [5]. The analysis of these parameters modifies the task of interpreting diagnostic image data from subjective and qualitative to objective and quantitative. This approach is called radiomics [6]. Radiomics is a new high-tech approach to the analysis of imaging data. In neurooncology, radiomics allows not

only to carry out differential diagnostics, but also to determine the prognosis of the disease [5, 7]. The task of radiomics is the transformation of diagnostic images into biomarkers and the identification of imaging phenotypes through a comprehensive study by methods of histogram, texture, and morphometric analysis in combination with multidimensional clinical data [8, 9].

MRI with visual evaluation of tumor characteristics on routinely used sequences is the gold standard for diagnosing PExT [10]. The possibilities of differential diagnosis of PExT on the basis of their MRI patterns have been studied in detail, however, reliable differentiation of these neoplasms based on MR semiotics is often difficult [11–13]. The use of radiomics has demonstrated a high prognostic value for differential diagnosis between different types of PExT, including for determining the degree of their malignancy.

Extracting quantitative data from multimodal medical images using advances in data mining and machine learning allows you to identify the relationship between the digital properties of a medical image and clinical data for use in diagnostic decision support systems [14, 15]. Radiomic analysis includes several stages: acquisition

and pre-processing of images, segmentation, feature extraction, and construction of a statistical model for the relationship between clinical data and visualization parameters. These steps can be performed sequentially with manual selection of data for the final model. In the case of applying the methodology of artificial intelligence based on deep learning, the simulation occurs without preliminary separation of stages. The information content of radiomic features must be evaluated on a validation set and only then can it be used for diagnosis and prediction.

The radiomic analysis should include all tumor tissue without involving the surrounding structures and the possible edema zone. Therefore, the first and, in many respects, the determining step in the analysis of a diagnostic image is high-quality segmentation. Segmentation can be carried out both manually and by semi-automatic or automatic methods. With manual segmentation, the variability in the interpretation of the formation boundary by different specialists can be quite significant [16], while the final analysis parameters depend on how well the tumor was highlighted [17]. The use of semi-automatic segmentation reduces the variability in establishing the tumor's margin compared to manual methods [18].

After region of interest (ROI) segmentation, quantitative and qualitative data are extracted from the selected area. These include: shape features, histogram, texture parameters and high-order features describing the statistical features of the image obtained using Fourier transforms and wavelet analysis [9, 19–24]. The construction of a predictive model is most often performed on the basis of deep learning using neural networks. In addition, common information technologies in radiomics are logistic regression, various types of decision trees [19]. Since a large amount of data is needed to achieve the reliability of the obtained statistical models, research in the field of radiomics is often multicenter. However, MRI may differ in scanning parameters, the impact of which on the final results must be taken into account [25]. The impact of imaging protocols on retrievable radiomic features has been analyzed in a number of studies [26–29]. These works have shown that image filtering is able to level out technological features, and differences in data acquisition parameters and moderate differences in MRI slice thickness do not affect the results of texture analysis.

2 Materials and Methods

2.1 Material and Used Features

The aim of the study is to compare the capabilities of visual semiotics and texture analysis of MRI for the differential diagnosis of PExT. The retrospective study included 66 patients with PExT, which were divided into two groups: training and validation. The training group included 39 patients (six men and 33 women) aged 33 to 62 years (median age 58 years). The validation group consisted of 27 patients (3 men and 24 women) aged 29

to 79 years (median age 59 years). Before surgical treatment, all patients underwent a standard MRI study using intravenous contrast (0.1 mmol/kg) on a tomograph with a magnetic field induction of 1.5 T. The scanning protocol included T2 weighted images (WI), T1 WI, FLAIR, and DWI (with subsequent ADC mapping). After contrasting, scanning was performed using a 3d sequence to obtain T1 WI (T1 CE). For each patient, about 20 images were obtained in various modes in which the tumor was observed.

The research included two directions.

1) Differential diagnosis of tumors based on visual evaluation of semiotics. Both qualitative (localization, shape, contour, structure, etc.) and quantitative (area and volume of the tumor, MR signal intensity) signs were evaluated.

2) Differential diagnosis of tumors based on textural parameters. Texture analysis was performed using the MaZda program [30, 31], which allows calculating the following groups of texture features: a) based on statistical characteristics, b) based on gradient parameters, c) based on the adjacency matrix, and d) based on run-length matrix. Before performing texture analysis, automatic segmentation of tumors was carried out using a convolutional neural network [32]. When performing texture analysis, the brightness of segmented images was normalized in the range $[\mu - 3\sigma, \mu + 3\sigma]$, where μ is the average value of the gray level, and σ is the standard deviation, Eq. (1):

$$Y_{i,j} = \frac{X_{i,j} - (\mu - 3\sigma)}{(\mu + 3\sigma) - (\mu - 3\sigma)}, \quad (1)$$

where $Y_{i,j}$ is normalized pixel brightness and $X_{i,j}$ is source pixel brightness.

Discriminant analysis was used as a method to perform statistical modeling based on the assessment of the cumulative contribution of various features to separate tumors.

2.2 Texture Features

As recent studies show, texture analysis has shown good results in identifying regions of interest in medical images, as well as in recognizing biomedical images and their further diagnostics [33–40]. Thus, in research [41] studied the problem of detecting pathologies in images of blood cells based on the analysis of textural features of various classes of initial images in different color subspaces; in research [42] the task of detecting lung or liver cancer; high accuracy of segmentation of fundus images showed in research [33, 43]. The results presented in research [44–47] indicate that the proposed technology of texture analysis is best suited for the analysis of X-ray images. The texture analysis used in research [40] showed that the proportion of correctly classified images ranged from 74% to 89%, depending on the selected area of interest. Most of the features used were based on run lengths feature. Below we present the

most common features used for informative feature selection (Eqs. (2–5)).

2.3 Histogram-Based Features

$$\text{– mean: } \mu = \sum_{i=1}^{N_g} ip(i); \quad (2)$$

where $p(i)$ is a normalized histogram vector (i.e. histogram whose entries are divided by the total number of pixels in ROI), $i = 0, 1, 2, \dots, N_g - 1$, N_g denotes the number of intensity levels;

$$\text{– variance } \sigma^2 = \sum_{i=1}^{N_g} (i - \mu)^2 p(i); \quad (3)$$

$$\text{– skewness } \mu_3 = \sigma^{-3} \sum_{i=1}^{N_g} (i - \mu)^3 p(i); \quad (4)$$

$$\text{– kurtosis } \mu_4 = \sigma^{-4} \sum_{i=1}^{N_g} (i - \mu)^4 p(i) - 3; \quad (5)$$

$$\text{– percentile-}n: i \text{ if } \sum_{k=0}^i p(k) \leq n,$$

where $n = 0.01, 0.1, 0.5, 0.9, 0.99$.

Gradient-based parameters. For the gradient feature calculation the following neighborhood for image pixel $x(i, j)$ is defined:

A	B	C	D	E
F	G	H	I	J
K	L	$x(i, j)$	N	O
P	Q	R	S	T
U	V	W	Y	Z

Based on this neighborhood, the absolute gradient value ($|Grad(i, j)|$) is calculated for each pixel (Eqs. (6–7)):

– for 5×5 pixel neighborhood:

$$|Grad5(i, j)| = \sqrt{(W - C)^2 + (O - K)^2}; \quad (6)$$

– for 3×3 pixel neighborhood:

$$|Grad3(i, j)| = \sqrt{(R - H)^2 + (N - L)^2}. \quad (7)$$

For the $|Grad5(i, j)| = |Grad3(i, j)|$ matrix of M elements (which contains absolute gradient values for ROI pixels), the gradient features are defined as follows:

– mean absolute gradient Eq. (8):

$$GrMean = \frac{1}{M} \sum_{i, j \in ROI} |Grad(i, j)|; \quad (8)$$

– variance of absolute gradient Eq. (9):

$$GrVariance = \frac{1}{M} \sum_{i, j \in ROI} (|Grad(i, j)| - GrMean)^2. \quad (9)$$

2.4 Co-Occurrence Matrix-Derived Parameters

The second-order histogram is defined as the co-occurrence matrix $h_{da}(i, j)$. When divided by the total number of neighboring pixels $R(d, \alpha)$ in ROI, this matrix becomes the estimate of the joint probability, $p_{da}(i, j)$, of two pixels, a distance d apart along a given direction α having particular (co-occurring) values i and j . Formally, given the image $f(x, y)$ with a set of N_g discrete intensity levels, the matrix $h_{da}(i, j)$ is defined such that its (i, j) th entry is equal to the number of times that $f(x_1, y_1) = i$ and $f(x_2, y_2) = j$, where $(x_2, y_2) = (x_1, y_1) + (d \cos \alpha, d \sin \alpha)$.

This yields a square matrix of dimension equal to the number of intensity levels in the image, for each distance d and orientation α . In MaZda, the distances $d = 1, 2, 3, 4$ and 5 pixels with angles $\alpha = 0^\circ, 45^\circ, 90^\circ$, and 135° are considered. The co-occurrence matrix-derived parameters computed by MaZda are defined by the equations that follow, where μ_x, μ_y , and σ_x, σ_y denote the mean and standard deviations of the row and column sums of the co-occurrence matrix, respectively. Thus, the co-occurrence matrix-based parameters are computed up to 20 times, for $(d, 0); (0, d); (d, d); (d, -d)$.

Co-occurrence matrix-derived features:

– entropy Eq. (10):

$$Entropy = - \sum_{i=1}^{N_g} \sum_{j=1}^{N_g} p(i, j) \log(p(i, j)), \quad (10)$$

– difference entropy Eq. (11):

$$DifEntrp = - \sum_{i=0}^{N_g} p_{x-y}(i) \log(p_{x-y}(i)), \quad (11)$$

– correlation Eq. (12):

$$Correlat = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_g} ij p(i, j) - \mu_x \mu_y}{\sigma_x \sigma_y};$$

$$p_{x-y}(k) = \sum_{i=1}^{N_g} \sum_{j=1}^{N_g} p(i, j), |i - j| = k, k = 0, 1, \dots, N_g - 1, \quad (12)$$

– inverse difference moment or homogeneity Eq. (13):

$$InvDfMom = \sum_{i=1}^{N_g} \sum_{j=1}^{N_g} (p(i, j) / (1 + (i - j)^2)). \quad (13)$$

2.5 Run Length Matrix-Based Parameters

Parameters based on run length matrix are calculated 4 times for each region of interest (for vertical, horizontal, 45-degree and 135-degree directions). $p(i, j)$ is the number of times a run of length j has grey level i . N_g is the number of grey levels and N_r is the number of runs.

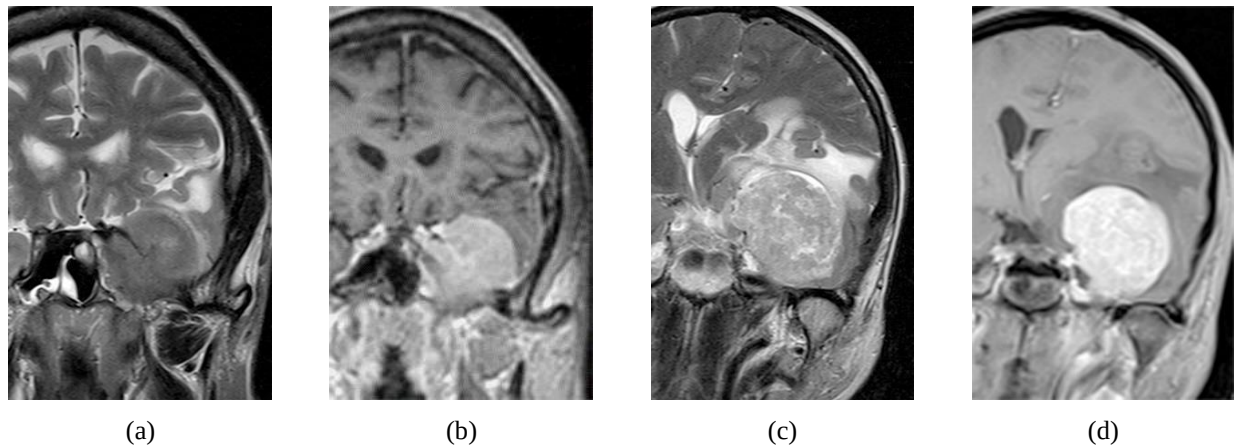


Fig. 1 Two meningiomas: malignant (a, b) and benign (c, d): T2 WI (a, c) and T1 CE (b, d). Differential diagnosis of these tumors, based on visual assessment of their signs, is difficult: both tumors are similar to each other, they have a rather heterogeneous structure and pronounced uniform contrasting. However, according to the results of histological examination, the first is malignant and the second is benign. Images dimensions: 200 px × 256 px.

Mazda definitions of run length matrix parameters are given below.

– short run emphasis inverse moments Eq. (14):

$$ShrtREmph = \frac{1}{n_r} \sum_{i=1}^{N_g} \sum_{j=1}^{N_r} \frac{p(i, j)}{j^2} = \frac{1}{n_r} \sum_{j=1}^{N_r} \frac{p_r(j)}{j^2}, \quad (14)$$

where $p_r(j) = \sum_{i=1}^{N_g} p(i, j)$, $n_r = \sum_{i=1}^{N_g} \sum_{j=1}^{N_r} p(i, j)$.

– grey level nonuniformity Eq. (15):

$$GLenNonUni = \frac{1}{n_r} \sum_{i=1}^{N_g} \left(\sum_{j=1}^{N_r} p(i, j) \right)^2. \quad (15)$$

3 Result and Discussion

According to the results of the reference test (histological examination of the surgical material): in the training group, benign meningiomas (M1) were diagnosed in 21 patients, malignant meningiomas (M2) in nine cases, the other nine formations were neurinomas (N).

The structure of detected tumors in the validation group: 15 of M1s, 6 of M2s, 6 of Ns. Classification of tumors based on semiotic features was difficult in some cases. Malignant tumors, in some cases, did not show typical MRI signs of malignancy (heterogeneity of structure, unevenness and lack of contrast) (Fig. 1) In some cases, with the localization of tumors in the region of the cerebellopontine angles, the differential diagnosis between benign tumors (benign meningiomas and neurinomas) was also difficult (Fig. 2).

Two statistical models were constructed based on the data of the training groups: using semiotic features (model 1) and based on textural parameters (model 2). The analysis of the diagnostic efficiency of the discriminant models consisted in calculating the operational characteristics of the tests: sensitivity, specificity based on the results of the validation of the models (Fig. 3). It can be noted that visual assessment of MRI data has a high sensitivity (73.3–100%) for benign

tumors, while the sensitivity for detecting malignant PET variants is only 50% (Fig. 3). Visual assessment of signs of tumors is not specific and subjective.

These results are consistent with world studies [48–50]. Visual assessment of MRI data has a high sensitivity (82.6–100%) for benign tumors (M1 and H), while the sensitivity for determining malignant variants of PET tends to zero (0–65.8%).

The model based on the signs of semiotics included the following signs: the average intensity of the MR signal, the minimum value of the measured diffusion coefficient, the volume of the tumor, the presence of necrosis zones, and changes in the underlying bone. The model built on the basis of textural features contains 20 parameters: gray level unevenness, features based on a matrix of series lengths that are significantly different for tumors of different histological types. These signs reflect the heterogeneity of the tumor structure.

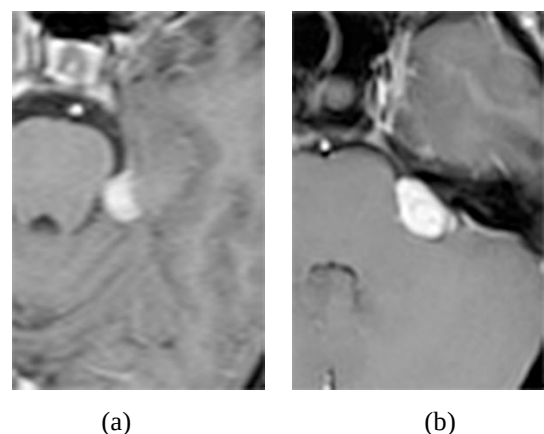


Fig. 2 T1 CE MRI images of meningioma (a) and neurinoma (b) of the cerebellopontine angles. Visual differentiation of neoplasms is difficult due to the similarity of signs: both tumors are widely adjacent to the meninges, their structure is homogeneous, contrasting is pronounced and homogeneous. Images dimensions: 100 px × 150 px.

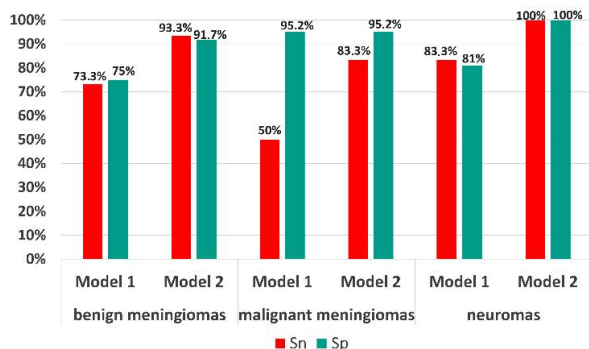
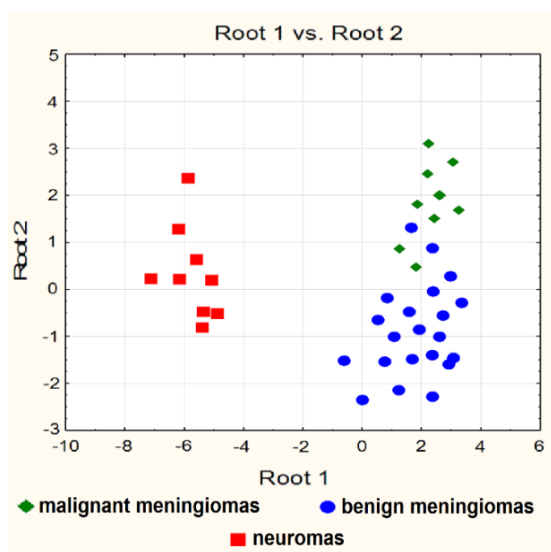
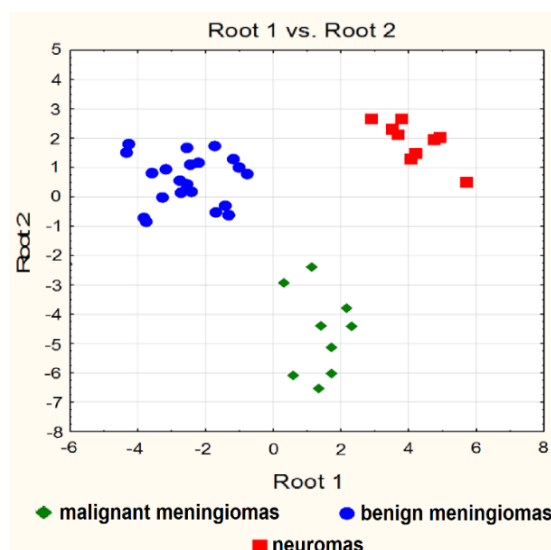


Fig. 3 Diagrams of sensitivity (Sn) and specificity (Sp) characteristics for various discriminant models based on the results of their validation.



(a)



(b)

Fig. 4 Feature distribution diagrams for models on training data (a) based on semiotic features, (b) based on histogram and texture parameters.

Differential diagnosis of meningiomas and neurinomas was carried out on the basis of histogram parameters. The informative features selected by discriminant analysis were characterized by the maximum contribution to the separation of tumors.

Diagrams (Fig. 4) clearly show the ability of discriminant models to separate tumors. The axes show the canonical roots of discriminant correlation analysis. There is an intersection of spaces for model 1 between benign and malignant meningiomas and a complete separation of tumors in model 2.

The superiority of the textural model of differential diagnosis is also confirmed by ROC analysis on validation. To build ROC curves, the data were converted in the format “true” (if the type of tumor identified by the model coincided with the results of the histological examination) “false” (if the simulation data differed from reality). The area under the curve for the texture model was 0.94, and for the model based on semiotics features it was 0.89 (Fig. 5).

For the practical use of the obtained statistical model for the classification of tumors based on textural features, it is necessary to use the discriminant classification function, which has the following form Eq. (15):

$$F = a_0 + \sum_{i=1}^{19} a_i x_i, \quad (15)$$

where x_i – features and a_i – coefficients, the values of which are presented in Table 1.

The discriminant function is calculated for each of the options (M1, M2, N). The tumor type is predicted based on which of the variants corresponds to the highest value of the discriminant function.

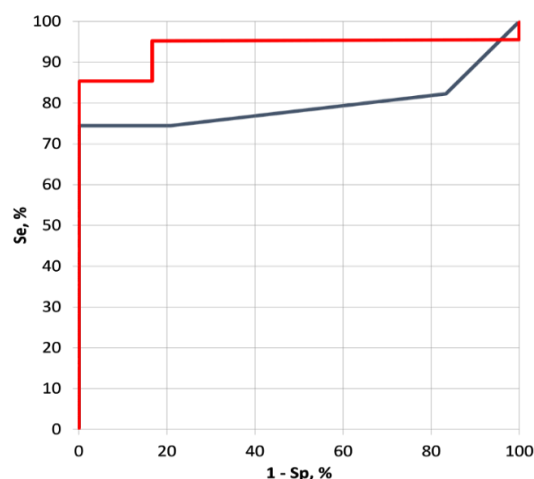


Fig. 5 ROC curves for two models of differential diagnosis on validation data: red – based on texture features, blue – based on MRI semiotics features. To build ROC curves, the data were converted in the format “true” (if the type of tumor identified by the model coincided with the results of the histological examination) “false” (if the simulation data differed from reality).

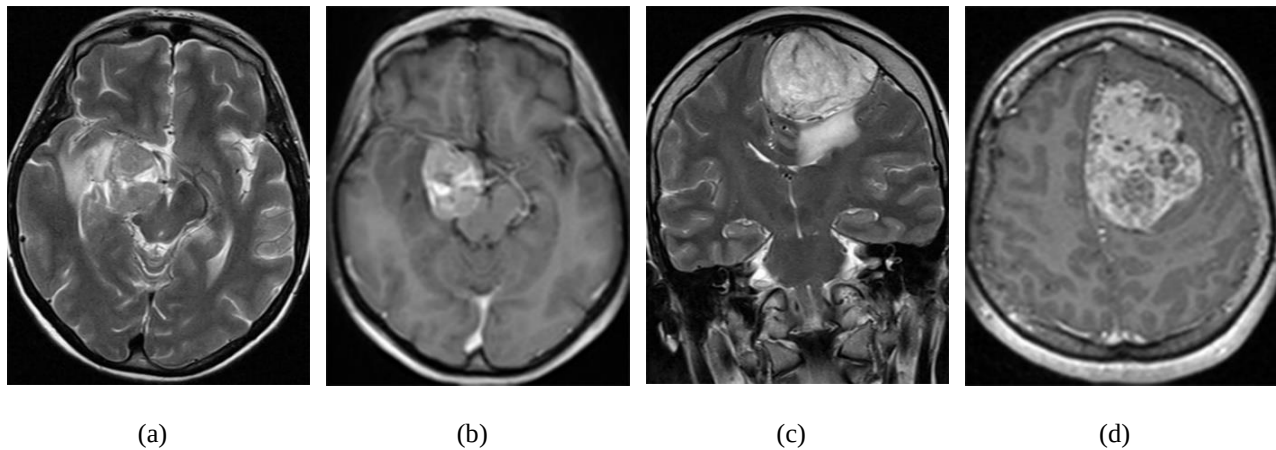


Fig. 6 Two meningiomas are shown: benign (a, b) and malignant (c, d): T2 WI (a, c) and T1 CE (b, d). The maximum value of the discriminant classification function for the first tumor indicated a benign meningioma, for the second formation it indicated a malignant meningioma. Images dimensions: 200 px × 256 px.

Table 1 Classification function coefficients of a discriminant model based on histogram and textural features for the differential diagnosis between benign meningiomas, malignant meningiomas and neurinomas.

i	Feature x_i			Coefficient a_i		
	Name	Scanning Protocol	MaZda feature	M1	M2	N
0	a_0			-153641	-146129	-166594
1	Sum of squares	T2	S(1, 0)SumOfSqs	-61786	-60247	-64529
2			S(5, -5)SumOfSqs	8518	8310	8946
3	Sum of variances		S(1, 0)SumVarnC	4144	4036	4332
4	Entropy sum		S(1, -1)SumEntrp	460974	449728	480528
5	Sum of averages		S(0, 5)SumAverg	-5187	-5085	-5444
6	Run length unevenness		135dr_RLNonUni	-3	-3	-3
7	Gray level unevenness		Vertl_GLevNonU	-5	-4	-5
8	Wavelet energy		WavEnLH_s-3	26	26	27
9	Entropy difference		S(2, -2)DifEntrp	5647	5304	5623
10	Inverse differential moment		S(5, 0)InvDfMom	-11028	-10480	-11018
11	Run length unevenness	T1	135dr_RLNonUni	-1	-1	-1
12	Wavelet energy	FLAIR	WavEnLL_s-2	1	1	1
13			WavEnHH_s-4	-23	-22	-24
14	Wavelet energy		WavEnLH_s-2	8	8	9
15	Sum of variances	ADC	S(0, 3)SumVarnC	2052	2004	2147
16	Run length unevenness		Horzl_RLNonUni	1	1	0
17	Histogram variance	T1 CE	Variance	8	7	8
18	Histogram skewness		Skewness	6117	5976	6373
19	Run length unevenness		135dr_RLNonUni	1	1	1

The results of the models are clearly demonstrated by the following clinical example (Fig. 6). In the world, 11 studies dealt with the differential diagnosis of

meningiomas, with 9 of them devoted to the differentiation between M1 and M2 (accuracy on average 88%) [51], and only one study devoted to the differential

diagnosis of meningiomas from neuromas (accuracy 96%) [52].

4 Conclusion

The possibilities of differential diagnosis of PExT by visual assessment of MRI images have been studied in detail, however, reliable differentiation of these neoplasms based on MR semiotics is often difficult. This is due to the insufficient information content of a number of signs, as well as the presence of the same or similar tomographic features in various types of tumors. Interest in studying the problem of PExT recognition based on the principles of radiomics is constantly growing, which is due to the desire to improve the diagnostic potential of MRI using the increase in computing power available to researchers, the improvement of the mathematical apparatus of machine learning and pattern recognition methods, among which the most promising is the texture analysis of tumors.

The results of the study showed that the analysis of standard MRI data based on radiomics shows a better

outcome in the differential diagnosis of PExT compared to visual assessment.

The proposed statistical model has high sensitivity and specificity for differential diagnosis between the three most common PExT: benign and malignant meningiomas and neurinomas. Modeling based on discriminant analysis demonstrated that textural features can be used for the differential diagnosis of PExT, and are highly informative, 33.3% superior to the model based on semiotics features. The use of quantitative indicators describing the structure of the tumor can improve the accuracy of differential diagnosis and is devoid of subjectivity.

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Disclosures

The authors declare no conflict of interest.

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Automatic Selection of the Optimal Zone for Laser Exposure According to the Fundus Images for Laser Coagulation

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Abstract. We analyzed a problem of extracting regions of interest (RoI) in eye-fundus images in the laser treatment for diabetic retinopathy (DR) using machine learning of deep neural networks aimed at higher-accuracy recognition of pathological and anatomical structures in the macula edema region. In this paper, we propose a method for automatic selection of the optimal zone of laser exposure based on images of the fundus for laser coagulation. Two neural networks were used to solve the problem. The first singled out anatomical objects in the fundus, and the second edema zone. The result was formed from the edema area, taking into account the location of anatomical objects on it. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: full convolutional neural networks; fundus imaging; macular edema; laser coagulation.

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1 Introduction

Diabetes mellitus is a common endocrine disease affecting all human organs. On the part of the visual apparatus, this manifests itself in the form of diabetic retinopathy (DR) [1–5]. Today, there are almost 400 million patients with diabetes in the world, and by 2035 the number is expected to increase to 592 million people [6]. The most dangerous manifestation of DR is macular edema. The walls of the retinal vessels become thinner and hemorrhages occur in the retinal area, leading to partial or complete loss of vision. According to the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR), with a duration of diabetes for more than 20 years, retinopathy is detected in 80–100% of cases, while diabetic macular edema (DME) develops in 29% of cases [7–8].

One of the methods used in medical practice for the treatment of DR is laser photocoagulation, during which certain areas of the retina are cauterized with a laser, stopping the spread of macular edema. There are no common systems for the treatment of diabetic retinopathy in the world that would provide a coagulation

plan with sufficient treatment efficiency, which is why many experienced doctors prefer manual laser guidance [9–10]. NAVILAS is a device of the German company ODOS [11, 12], which allows you to perform laser coagulation according to a pre-formed plan. The plan is formed manually, placing a hexagonal pattern in the area of influence. The limited patterns do not suit physicians [11, 13, 14], and they return to the use of old equipment with manual laser guidance, such as Valon [13].

To form a plan, it is necessary to determine which zone can be fired upon, as well as which zone is optimal, in the sense that by eliminating areas that are inappropriate for exposure, the number of laser shots and, accordingly, the costs of the substance used are reduced, and the impact on the fundus is also reduced, which should improve the quality of treatment.

In the modern world, neural network algorithms are used to solve data mining problems. With the development of technology, convolutional neural networks have found wide application in the tasks of image and video data processing. In particular, in biomedicine, neural networks allow solving problems of

semantic segmentation, search and recognition of objects in an image [15, 16], for example, determining the area of lung damage by the SARS-CoV-2 virus [17] or cancerous tumors of the human brain [18].

In this paper, we propose a method for the formation of the optimal zone of laser exposure based on the selection of the zone of macular edema and anatomical objects in the images of the fundus. This method reduces the traumatic nature of laser photocoagulation surgery.

2 Materials and Methods

2.1 Selection of the Optimal Zone for Laser Exposure

Two neural networks were used to form the laser impact zone. The first network allows us to select anatomical objects in the images of the fundus. The second network highlights the edema zone from the fundus images.

Algorithm for selecting the optimal zone of laser exposure:

1. Using the first neural network, anatomical objects are highlighted in the image.
2. Using the second neural network, the edema zone is highlighted in the image.
3. The resulting neural network segmentation maps are a probability distribution requiring post-processing. At this stage, the map is also binarized.

The edema zone is corrected taking into account the anatomical landmarks located on it (the masks of anatomical landmarks are subtracted from the mask of the edema zone), thus a preliminary mask of the laser exposure zone is obtained.

4. The resulting mask of the optimal zone of laser exposure is obtained from the preliminary mask by automatic processing it using the morphological functions of erosion and dilation to clarify the boundaries of macular edema with kernel size 7×7 . As well as processing with a median filter to smooth out the unevenness of the mask with kernel size 7×7 .

All neural network architectures used are fully convolutional neural networks. This type of neural networks is best suited for solving semantic segmentation problems because: the entire image context is taken into account, segmentation is performed in one pass, they are well known and widely popular.

2.2 Augmentation

The popularity of using full convolutional neural networks in semantic segmentation problems is determined by a number of factors. Thus, neural networks have a good generalizing ability and are able to take into account the entire context of the image, which allows them to be used in cases where other methods show worse results. However, neural networks also have limitations that directly depend on the characteristics of the training data set. In this regard, the use of neural networks in biomedicine faces a number of specific problems.

1. Due to patient privacy policies and the complexity of data labeling, as well as the requirements for the qualification of a specialist who performs labeling, data sets are often small [19]. The use of augmentation methods can partially compensate for the insufficient size of data for training [20]. In particular, for example, in tasks related to the analysis of biomedical images, the method of elastic data augmentation [21] is popular and effective: rotation at an arbitrary angle, reflections, and elastic deformation.

2. A frequent problem in tasks related to medicine, a pronounced problem is the imbalance of classes [22]. Solving the problem of class imbalance in the training dataset is non-trivial, but there are methods to smooth out the impact of this problem [23, 24]. For example, in the case of fundus image processing, samples often contain classes that are rarely found on most images, and, accordingly, the relative volume of these classes is extremely small. This problem of class imbalance should be taken into account when designing neural networks. Various kinds of algorithms should be applied that can level the influence of this problem [25, 26].

3. The most difficult problem is the low quality of data labeling, which is the result of the low qualification of the specialist who did the labeling [27]. This problem is extremely difficult to identify and eliminate at the training stage.

The peculiarities of our problem are the fact that the original data are unbalanced, the number of images is small, and the labelling does not exactly match the actual location of the objects.

There are a number of works devoted to solving this problem, however, they are mostly highly specialized and consider only the segmentation of images into one class, for example, the class of blood vessels [28] or exudates [29]. In paper, the problem of segmentation of fundus images into several classes is considered, which is relevant for creating a decision support technology for a doctor in the diagnosis and treatment of diabetic macular edema [30].

In accordance with the problems mentioned earlier, an important step in the application of neural networks is data preparation: smoothing out the influence of a small amount of training sample and class imbalance. In this work, the following image augmentation techniques were used: reflection, rotation by a random angle (from -30° to 30°), random shift, and elastic transformation. The use of augmentation allows us to successfully train a neural network to perform semantic segmentation, as well as to deal with network retraining when using small data sets.

2.3 Segmentation Anatomical Objects

For step 1 of the algorithm, the neural network described in Ref. [31] was used.

Based on the Unet network, the ResNetUnet, DenseNetUnet and XceptionUnet networks were built, where one of the ResNet, DenseNet, or Xception networks, respectively, was used as an encoder. The weights of the pretrained networks were used to initialize the encoder and were fixed for the duration of the training.

The ResNetUnet architecture uses the ResNet-101 network [32] pretrained on the ImageNet data set [33] as a feature encoder. The features of the ResNet architecture include its depth. It was the first architecture that allowed researchers to train neural networks with more than 20 layers. Also in this architecture, skip-connection (a modification of the architecture that allows the signal to pass through the network by skipping one or more layers) was used for the first time to prevent the fading gradient problem.

The DenseNetUnet architecture uses the DenseNet-169 network [34] as a feature encoder, which was pretrained on the ImageNet dataset. The DenseNet architecture learns well on small datasets and proposes to connect all layers using skip-connection within one building block of the network.

XceptionUnet uses the Xception-65 network [35] as a feature encoder, which was pretrained on the ImageNet dataset. The features of this architecture of the neural network include the use of a combination of pointwise (point convolution with a kernel size of 1×1) and depthwise (spatial convolution independently applied to each channel) convolutions instead of classical convolution. This replacement allows to reduce the number of trained parameters without affecting the accuracy of the network.

For the experiment, a dataset was used, consisting of 115 fundus images, which were labeled into 8 classes: optical disc (OD), macula (M), blood vessels (BV), hard exudates (HE), soft exudates (SE), new coagulates (NC), pigmented coagulates (PC), and hemorrhage (H). Images have dimensions of $1024 \text{ px} \times 1024 \text{ px}$ and 3 rgb color channels.

Neural networks are built and trained using the TensorFlow library. The following parameters were used in training:

- Input size: $1024 \text{ px} \times 1024 \text{ px} \times 3$.
- Number of epochs: 120.
- Loss function: FocalLoss [36].
- Optimizer: Adam [37].
- Learning rate: 0.003.

The specified number of epochs is the maximum value of possible training epochs. Each neural network had its own optimal epoch, at which training stopped if the results did not improve within 20 epochs. During the research, it was found that the XceptionUnet network shows the highest accuracy among those studied.

2.4 Segmentation Edema Zone

To search for a suitable neural network architecture for the second point of the laser exposure area selection algorithm, in this work, such neural networks as Unet, Unet++, Manet, Linknet, Feature Pyramid Network (FPN), Pyramid Scene Parsing Network (PSPNet), Pyramid Attention Network (PAN), and Deeplabv3 were used.

All neural networks used in this work are based on the resnet-34 network pre-trained on images from the ImageNet dataset. Using a pre-trained neural network allows us to train your own networks faster, because

such networks are already capable of extracting a huge number of features from images.

Unet [38] is one of the first fully convolutional neural network architectures successfully applied to solve the problem of semantic segmentation of biomedical images. The Unet architecture can be represented schematically in the form of the letter U. This architecture consists of two parts, an encoder that acts as a classifier or feature extractor and a decoder that maps the image segmentation from the features extracted by the encoder.

Unet++ is an improved version of the Unet architecture, which was also developed for use in tasks of semantic segmentation of biomedical images [39]. The main change in this architecture is the replacement of the links between the encoder and decoder with small intermediate networks. Unlike simply concatenating the outputs of the encoder layers to the outputs of the decoder layers, the use of such intermediate networks improves the internal representation of the features of the neural network in such a way that it allows the decoder to more accurately build a segmentation map.

The architecture of the MAnet neural network was developed to solve the problem of semantic segmentation of images obtained as a result of scanning the organs of the liver and brain [40]. A feature of this neural network is the use of the attention mechanism. This mechanism models the human ability to focus on an object or area of interest. Thus, this mechanism allows the neural network to pay attention to certain areas, features, channels, depending on the area under consideration. Accounting for such information allows us to train more accurate neural networks. According to the study, the addition of the attention mechanism improves the accuracy of semantic segmentation in comparison with the neural network of the Unet and Unet++ architectures.

The Linknet architecture is similar to the Unet architecture with the exception of some changes [41]. So, in the original Unet, the decoder and encoder are interconnected by simple links. These links extend the outputs of the encoder with the outputs of the decoder. In the Linknet architecture, it is proposed to use the residual connection proposed by the authors of the Resnet architecture. Thus, the outputs of the decoder are added to the outputs of the encoder. According to the study conducted by the authors, this modification improves the accuracy of the final model.

The FPN architecture is also similar to the Unet architecture [42]. Its feature is how the outputs of each decoder block are used. In Unet, the final segmentation map is the output from the last layer of the neural network. Whereas in FPN the final segmentation map is made by weighted summation of the output of each decoder block. This feature to a greater extent allows us to better take into account the general context of the image at different scales, as well as deal with overfitting of the neural network.

The architecture of PSPNet is fundamentally different from those considered earlier [43]. The typical U-shaped architecture does not apply here. So PSPNet consists of a convolutional neural network (CNN) which also acts as

a feature encoder. Further, the features from the last layer of the CNN fall into a special module, in which convolutions of different sizes are applied to the features. The convolution data performs feature localization. Next, each convolution output is upsampled to the size of the original image using an upsampling layer. After that, all layers are concatenated together with the encoder feature map. For this tensor, the final convolutional layer is applied, which forms a segmentation map.

The PAN architecture [44] similarly to MAnet uses the attention mechanism to improve the accuracy of the neural network. While I use Position-wise Attention Block (PAB) and Multi-scale Fusion Attention Block (MFAB) in MAnet, Feature Pyramid Attention (FPA) is used in PAN architecture. This block is designed to improve the perception of the attention block itself by building a structure inside the FPA similar to that implemented in the FPN network architecture. Thus, the FPA block allows you to highlight features from different scales, thereby making up for the lack of classical mechanisms for attention to pixel information.

The Deeplabv3 [45] architecture also consists of an encoder and a decoder. However, unlike all previous architectures, the main idea of this architecture is to replace conventional convolutional layers with sparse convolution layers (atrous convolution). These layers are designed to help train the neural network to better understand the context of the image.

For the experiments, we used a set of 50 fundus images (with dimensions of $1024 \text{ px} \times 1024 \text{ px}$ and 3 rgb color channels) labeled by an ophthalmologist. This dataset differs from the dataset used in the segmentation of anatomical objects. Fig. 1 shows an example of an image of the fundus and Fig. 2 shows its markings.

So, using augmentation methods, it allowed us to expand our data set to 5000+ images. The initial data set was divided into training and test sets in a ratio of 4 to 1. Thus, 40 fundus images represented the training set and 10 images represented the test set.

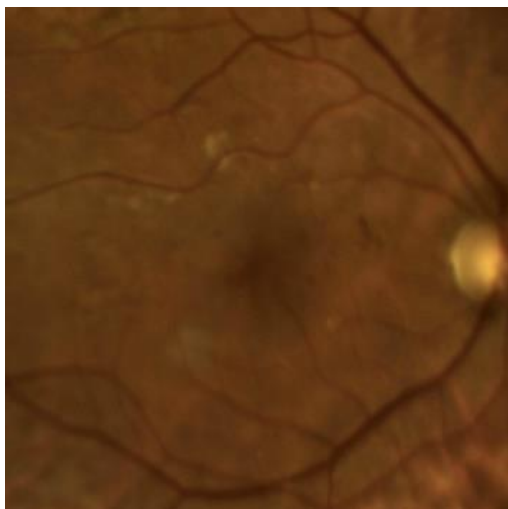


Fig. 1 Source image ($1024 \text{ px} \times 1024 \text{ px}$).

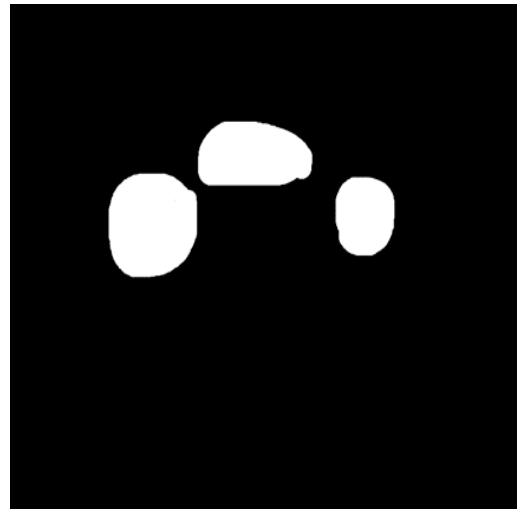


Fig. 2 Binary segmentation mask for macular edema ($1024 \text{ px} \times 1024 \text{ px}$).

All neural networks were trained with the same parameters:

- Input size: $1024 \text{ px} \times 1024 \text{ px} \times 3$.
- Number of epochs: 150.
- Loss function: Cross Entropy.
- Optimizer: Adam.
- Learning rate: 0.001.

3 Result and Discussion

During the experiments, four neural networks were selected that have the highest accuracy according to the f1 metric. Table 1 presents the results of this experiment.

Table 1 Training results for various neural network architectures.

Architecture	f1
Unet	0.584
Unet++	0.562
MAnet	0.508
Linknet	0.575
FPN	0.438
PSPNet	0.399
PAN	0.512
DeepLabV3	0.478

The following networks became the best by the metric: Unet, Unet++, Linknet, PAN. Further research was carried out with them. In the course of the research, it was found that if, in addition to the image of the fundus, a map of objects (optical disk, macula, vessels, etc.) is submitted to the input of the neural network, this will improve the accuracy of the model when highlighting the edema area. The objects were selected using a neural network from the first step of the algorithm for selecting

the area of the laser impact zone. The results of the corresponding experiment are presented in Table 2.

As can be seen from the results in the Table 2, for the three neural networks, the accuracy of edema area selection is improved. Further research will be carried out with the training of neural networks on images of the fundus combined with a map of objects. For fundus images, some researchers use a special kind of preprocessing, in which the original fundus image is added (weighted sum) to the same image processed using a Gaussian filter with a large kernel. Fig. 3 shows source image and Fig. 4 shows an example of applying this preprocessing to one of the images from the original dataset. Table 3 presents the results of an experiment that checks the effect of this preprocessing on neural network training in terms of the f1 metric.

The results of the conducted research showed that the use of such preprocessing does not have a clear positive effect on the accuracy of the neural network.

In order to obtain reliable results of the experiment, k-fold cross-validation was carried out. The data set was randomly divided into 5 non-overlapping groups so that 40 images were included in the training set, and 10 of them were included in the test set.

Table 4 shows the average result of the experiment in terms of precision, recall, f1 metrics. To evaluate each group, the best epoch in terms of the f1 metric was taken.

Table 2 Results of the experiment to check the effect of adding map of objects (f1 metric).

Architecture	Source image	Combined images
Unet	0.584	0.620
Unet++	0.562	0.632
Linknet	0.575	0.608
PAN	0.512	0.446

Table 3 Results of an experiment to test the effect of fundus image preprocessing (f1 metric).

Architecture	Source image	Preprocessed image
Unet	0.620	0.609
Unet++	0.632	0.626
Linknet	0.608	0.594
PAN	0.446	0.468

Table 4 Results of cross-validation (f1 metric).

Architecture	Linknet	PAN	Unet	UnetPlus Plus
precision	0.728	0.674	0.648	0.716
recall	0.565	0.533	0.470	0.592
f1	0.634	0.594	0.544	0.647

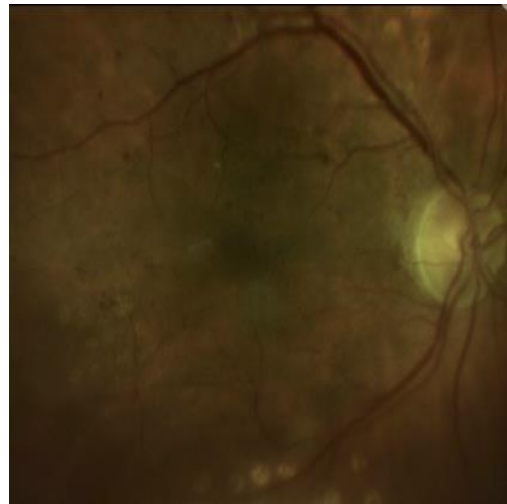


Fig. 3 Source image (1024 px × 1024 px).

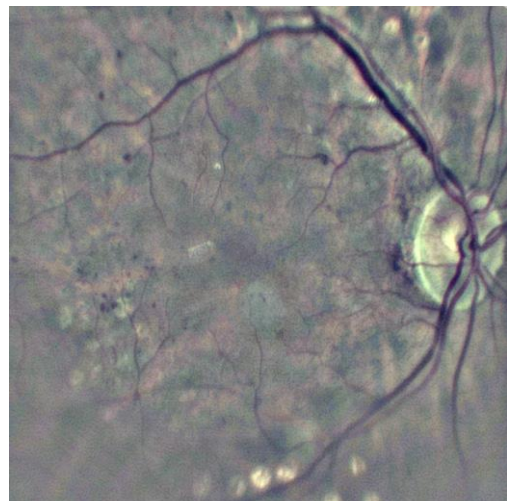


Fig. 4 Image after preprocessing (1024 px × 1024 px).

Thus, according to the results obtained, the most accurate model turned out to be Unet++. Fig. 5 shows an example of the results of semantic segmentation of images.

As mentioned earlier, when working with biomedical data, there is a problem of inaccurate labeling. In this work, the labeling problem is due to two factors. The marking of anatomical objects in the fundus is not absolutely accurate at the pixel level. For example, the edge of the vessels is inaccurately segmented, there is a small group of pixels that belong to the edge, but not marked by the expert. Also, the labeling of the edema area is not accurate due to the problem that the edema itself is not always visible in a planar image. For this reason, the ophthalmologist was guided by OCT data when marking, so the resulting marking is not always correct in the area where the edema is not clearly expressed.

With the help of augmentation techniques, this problem was partially solved using the generalizing ability of the neural network. Also, due to the specificity of this task, a direct comparison with the results of other studies cannot be correctly carried out.

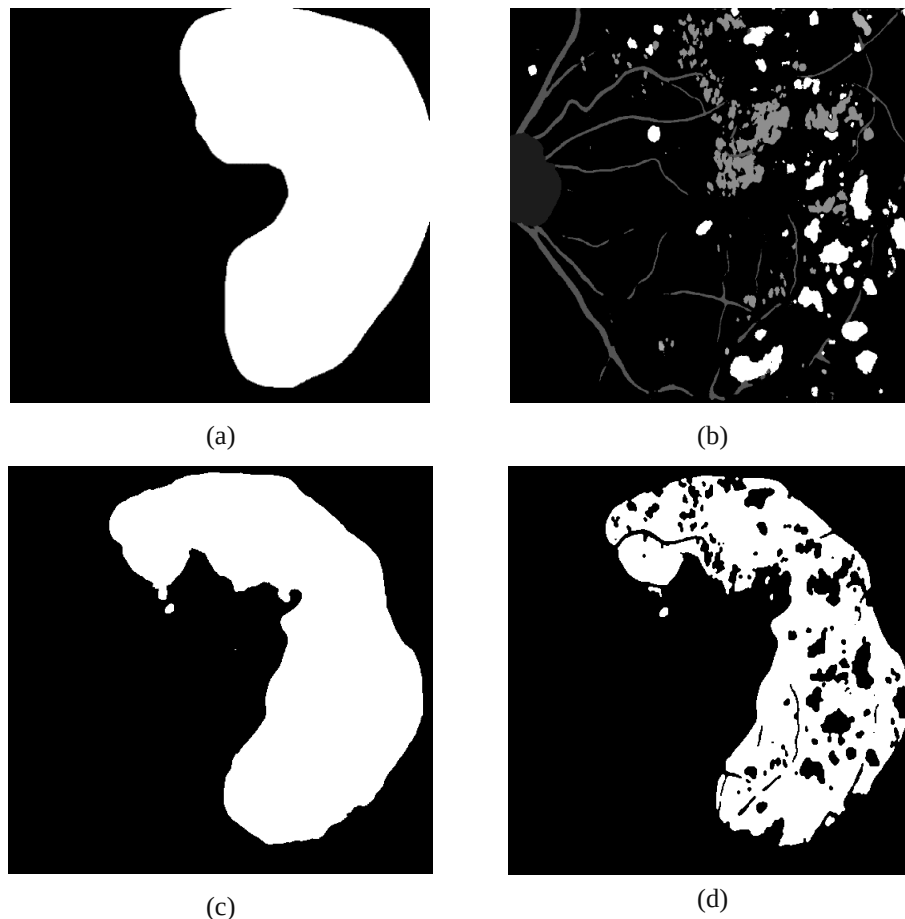


Fig. 5 Results of predictions of the area of macular edema by the neural network (1024 px × 1024px): (a) expert labels, (b) prediction of the neural network of the object map, (c) results of predictions of the neural network of the edema area, and (d) processed result.

Both the formulation of the problem itself and the initial data differ. This work is part of a semi-automatic system for creating a coagulation plan. The results obtained in the course of the work were subjected to expert analysis by an ophthalmologist and were considered sufficient for use in the system for constructing a coagulation plan. The hypothesis was confirmed that despite the weak severity of edema in the images of the fundus, neural networks are able to highlight it.

Further research will be aimed at improving the accuracy of segmentation of the fundus regions.

4 Conclusion

In this work, a method was developed and investigated for automatic selection of the optimal laser exposure zone from the fundus images for laser coagulation, based on the use of two neural networks. An important step

presented in this paper is the selection of the area of macular edema.

As a result of the research, it was shown that the use of the Unet++ model to highlight the edema area gives the highest accuracy relative to other architectures considered in the framework of the work. improve the accuracy of differential diagnosis and is devoid of subjectivity.

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Disclosures

The authors declare no conflict of interest.

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In Vitro Study of Tetracycline Penetration into the Enamel and Dentin of the Tooth by Fluorescence Spectroscopy

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Abstract. With the use of tetracycline in childhood, as well as with intrauterine or ectopic exposure before teething through the gums during mineralization or calcification of the teeth, the antibiotic binds to calcium ions in the teeth. This results in permanent staining of the teeth. In dentistry, the term "tetracycline teeth" even arose. During teething and exposure to light, calcium-bound tetracycline oxidizes, causing the enamel to change color from fluorescent yellow to brown. In this study, using fluorescence spectroscopy, it was found that tetracycline exhibits characteristic fluorescence peaks in the dentin and in the enamel of the teeth after exposure of the antibiotic solution to the extracted teeth *in vitro*. Thus, it was shown that in non-carious fully mineralized human dental tissues, tetracycline penetrates quite well into the dentin matrix and, to a much lesser extent, into the enamel matrix, retaining its fluorescence. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: enamel; dentin; human teeth; tetracycline hydrochloride; fluorescence spectroscopy; diffuse reflection spectroscopy.

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1 Introduction

Optical methods in clinical practice, which have been actively developed in recent years, are of great importance and great prospects both for the early diagnosis of various diseases and as methods of specific effective treatment [1, 2]. In dentistry, optical methods, in particular fluorescent, are used for aesthetic rehabilitation of teeth, diagnosis of caries, and detection of tartar in the prevention of periodontal disease, etc. [3]. The color of the teeth is determined by the absorption, scattering, and transmission of ambient light inside the tooth, the autofluorescence of the teeth is based on the presence of endogenous fluorophores found in the enamel and dentin [4]. Early diagnosis of caries by blue laser-induced autofluorescence is possible [5]. Occlusal,

secondary, and proximal dental caries were assessed using the Qraycam and Qraypen clinical systems based on laser-induced fluorescence [6].

A decrease in the intensity of fluorescence in the teeth was revealed, which effectively correlates with the loss of minerals inside the lesion, which can be used to effectively detection and monitor the smallest changes in demineralization / remineralization in early carious lesions without carious cavities [7].

Using fluorescence, it is possible to visualize the localization of pathogenic microorganisms of the oral cavity (*Candida albicans*, *Porphyromonas gingivalis*, *Streptococcus mutans*) and teeth, but the identification of each pathogen by this method is unlikely [8]. Registration of red fluorescence, which comes from porphyrin-induced metabolites produced by

microorganisms in the oral cavity, provides diagnostic of caries, dental plaque and tartar [9]. Raman spectrometry can be used to detect molecular structural changes responsible for laser-induced tooth fluorescence to improve the prediction of chemical whitening efficacy [10]. Autofluorescence imaging with 1% toluidin blue staining is effective as a method for diagnosing and detecting neoplasms of the oral mucosa [11]. Using wide-field multiwavelength fluorescence imaging and spectral analysis of the main components, various anatomical and morphological features of the periodontium, gum tissues, alveolar bone, cementum, and periodontal ligaments were determined [12].

Currently, new dental materials are being created for the restoration and restoration of dental tissue, which have similar fluorescent and the opalescence properties, along with shade and color [13, 14]. In dental composites and ceramics, fluorescence is achieved by incorporating materials containing rare earth luminescence centers to look more beautiful and lively [15]. However, many factors in the process of human life affect the color and fluorescence of teeth and restorative materials in different ways, for example, aging, temperature, whitening, bone grafting or bone augmentation, implant placement, taking various pharmaceuticals, and antibiotics, etc. [16]. The lack of knowledge about this matter makes more and intensive studies to be done. So, one of the biologically active drugs that actively accumulates in hard tissue is tetracycline. This broad-spectrum antibiotic may cause discoloration and hypoplasia of developing teeth in a growing child [17].

For identification and determination of tetracycline in aqueous solutions, an effective approach is a combination of adsorption pre-concentration followed by chromatographic [18] or luminescent detection [19]. An efficient method for the solid-phase luminescent determination of tetracycline using systems of chemically modified silica has been developed [20].

In recent years, organometallic scaffolds for the detection of antibiotics in food and the environment have been effectively studied. Using preliminary adsorption pre-concentration, sensitive responses to a number of tetracycline antibiotics (tetracycline, chlortetracycline and oxytetracycline) with detection limits of 0.28–0.30 μM have been obtained [21, 22].

A water-resistant carboxy-functionalized organometallic europium skeleton has shown effectiveness as a double reacting fluorescent sensor for tetracycline antibiotics (oxytetracycline, tetracycline, doxycycline) and dihydrogen phosphate detection [23]. This sensitive and selective tetracycline antibiotics sensing can be used to detect latent fingerprints on various surfaces. Since tetracycline is widely used in animal husbandry, antibiotic residues can be found in animal products. Fluorescence probing using Zn^{2+} as sensor probes detected tetracycline in a complex sample of chicken broth [24]. For rapid and specific detection of tetracycline residues in milk, copper-doped zinc sulfide

quantum dots were used as ratiometric fluorescent probes [25].

The use of antibiotics in young children is widespread and can lead to adverse dental health outcomes, including staining, developmental defects, and caries. Therefore, the development of simple and sensitive methods for the rapid screening of biologically active substances, for example, tetracycline in complex biological samples, is of great importance.

The review [26] described a dose-response relationship between exposure to tetracycline doses $>20 \text{ mg/kg}$ per day and tooth staining. Early childhood doxycycline exposure (at any dose) has not been associated with tooth staining, dental caries, or enamel defects. Further research is needed to determine the effect of antibiotics on oral tissues. The present study discusses whether tetracycline can be incorporated into the crystal lattice in fully mineralized enamel and dentin. To do this, tetracycline was qualitatively determined in the enamel and dentin of extracted human teeth *in vitro* using luminescent spectroscopy.

2 Materials and Methods

The material of the study was human teeth removed for orthodontic indications in a dental clinic. The penetration of tetracycline hydrochloride was studied on non-carious fully mineralized human teeth, namely dentin and enamel. For the study of enamel, a whole tooth was taken. To study the dentin, the teeth were cut with a diamond disk into longitudinal cuts. The samples were placed for 20 s in 35% phosphoric acid. To clean the surface from saw cut products, the samples were placed in a Techsonic UD100 SH-45 L ultrasonic bath with water for 10 min, and wiped with a brush dipped in ethanol. The samples were air dried for several days. The thickness of the sections (samples) of the teeth was measured with a micrometer at several points of the sample and averaged. The accuracy of each measurement is $\pm 10 \mu\text{m}$. The mean thickness of the sections was $0.52 \pm 0.08 \text{ mm}$ ($n = 3$, n is the number of samples). In total, three sections from different teeth and five whole teeth were examined in the experiment. The quality of cleaning from saw cut products and the study of morphology were carried out by scanning electron microscopy (SEM) on a Mira II LMU electron microscope (Tessan, Czech Republic) in the secondary electron recording mode (at an accelerating voltage of 30 kV). To do this, the samples were fixed on a special carbon substrate (carbon tape) and their surface was coated with gold. The diameter of the dentinal tubules was determined using scanning electron microscopy data.

As a fluorescence agent, we used a pharmaceutical preparation – tetracycline hydrochloride (manufacturer: Biochemist AO, Russia), with a concentration of tetracycline hydrochloride solution $C = 1.1 \cdot 10^{-4} \text{ mol/l}$. Working aqueous solutions of tetracycline hydrochloride were acidified by adding 1 drop of hydrochloric acid to $\text{pH} = 5$, since tetracycline solutions are unstable in alkaline and neutral media. Tetracycline can emit fluorescence when excited by ultraviolet light [27]. The

absorption spectrum of the obtained solution was studied on a two-beam spectrophotometer Shimadzu UV-2550 (Japan) in the range of 200–800 nm in a quartz cell with a thickness of 1 cm. The light source was a halogen lamp with radiation filtering in the investigated spectral range. All measurements were carried out at room temperature ($\sim 25^\circ\text{C}$) and normal atmospheric pressure. Diffuse reflectance and total transmittance spectra of tooth samples were recorded on the same spectrophotometer using a solid sample attachment. The conversion of diffuse reflectance spectra into absorption spectra was carried out using the Kubelka-Munk algorithm built into the software of the spectrophotometer. To study the penetration of the agent into the enamel and dentin of a human tooth, the test samples were placed in a Petri dish, with an aqueous solution of tetracycline hydrochloride. We previously determined the time of complete staining of *ex vivo* dentin samples of thickness 0.52 ± 0.08 mm as 5.0 ± 0.4 h [28]. Therefore, the samples were kept in the agent solution for 5 h. Before measurements, the samples were rinsed with distilled water to remove agent residues from the surface. The fluorimetric determination of tetracycline was carried out on an RF-5301PC spectrofluorimeter (SHIMADZU, Japan) using an attachment for solid samples with the following parameters: excitation light wavelength (λ_{ex}) was 376 nm, selectable spectral bandwidth of monochromators ($\Delta\lambda_{\text{ex/emission}}$) = 5/5 nm, scanning speed

was medium, spectral measurement range 220–800 nm with a step of 1.0 nm. A xenon lamp served as the light source. The scheme of the experiment is shown in Fig. 1. Photographs of human teeth samples were taken on a video densitometer “SORBFIL” (Russia) with an ultraviolet lamp with a maximum of emitting spectrum at 365 nm. Photos were taken on the smartphone “Iphone XS Max” (USA).

3 Results and Discussion

The human tooth consists of a root, a neck covered with cement, and a crown covered with enamel – the hardest tissue of the tooth. Cementum is a mineralized tissue consisting of calcified intercellular substance that covers the tooth in the area of the root and neck.

The morphology of enamel and dentin was studied using scanning electron microscopy (Fig. 2). Enamel is formed from prisms, which are located at different angles to the surface. Down the enamel is dentin (in the crown and root of the tooth). Enamel and dentin are mineralized connective tissue. A feature of dentin is the presence of dentinal tubules with a diameter of 2 to 5 μm , penetrating the entire thickness of the dentin and supporting cytoplasmic processes of odontoblasts – cells located on the periphery of the pulp. The number and diameter of the lumen of dentinal tubules are different in different parts of the dental organ [29, 30].

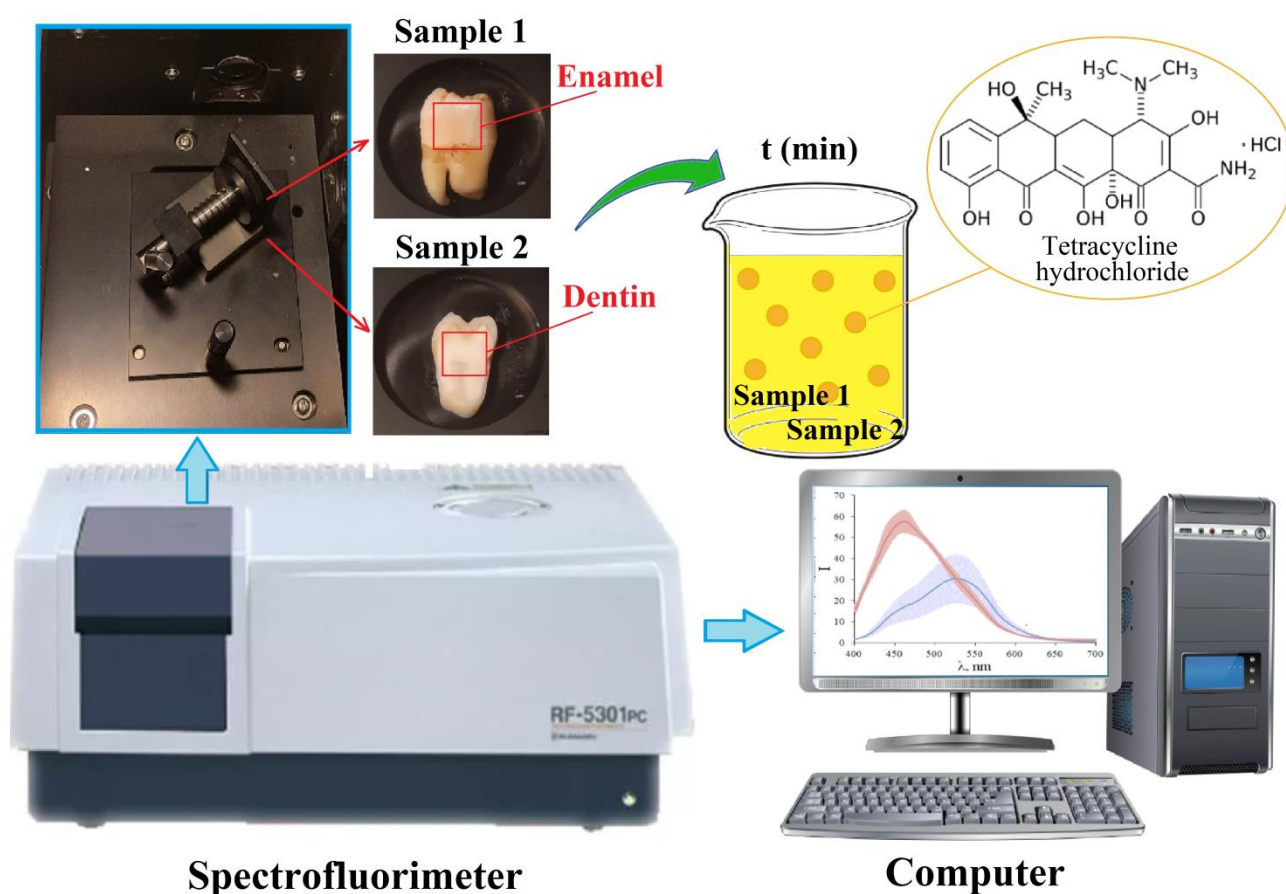


Fig. 1 Experimental scheme for measuring fluorescence spectra using an attachment for solid samples.

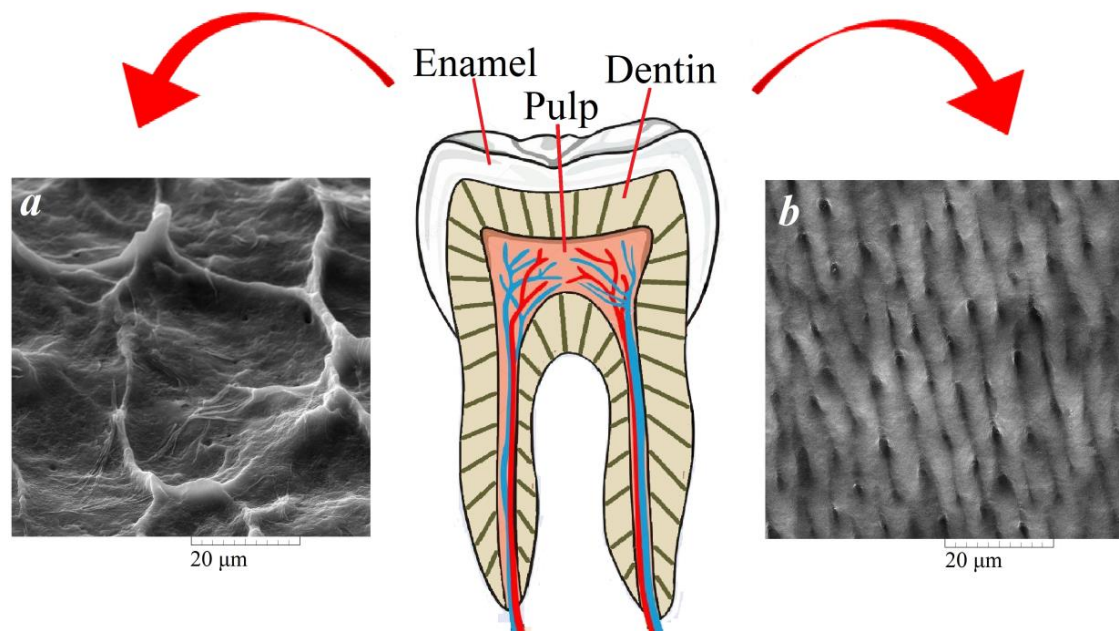


Fig. 2 Electronic micrographs (SEM) of a longitudinal section (sample) of a human tooth: (a) enamel (magnification $\times 5k$), (b) dentin (magnification $\times 5k$).

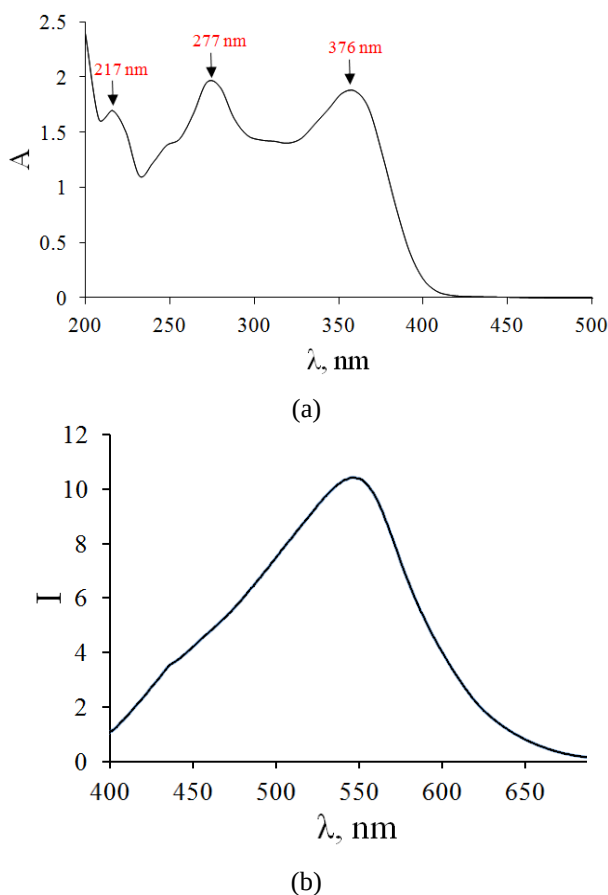


Fig. 3 Spectra of tetracycline aqueous solution: (a) absorption spectrum, (b) fluorescence spectrum at an excitation wavelength of 376 nm.

Regardless of how the light hits the outer surface of the tooth, pulp illumination is always effective due to the sinuous shape of the light guides, which are formed by

enamel prisms and structures of dentinal tubules. The waveguide effect is much more pronounced in dentin than in enamel [31].

From an optical point of view, dentinal tissue can be attributed to optically turbid media in which the shape of the diffuse reflectance and total transmittance spectra correlate over the entire studied range (200–800 nm). The optical properties of the tooth are mainly determined by dentin due to its bigger thickness than for enamel. Reflection of light by periodic structures of dental tissues provides information about those structures.

An absorption spectrum of tetracycline solution was obtained, which has characteristic maxima in the UV region of the spectrum at 220 nm, 277, and 376 nm (Fig. 3(a)). The fluorescence spectrum of the tetracycline solution has a pronounced peak at 550 nm at an excitation wavelength of 376 nm (Fig. 3(b)).

Endogenous chromophores determine measured diffuse reflectance and total transmittance spectra of tissues, from which absorption spectra can be reconstructed using the Kubelka-Munk algorithm. Chromophores in the dentin are protein-like molecules, amino acid residues, DNA, NADH-H, and partly collagen and elastin, causing the diffuse reflectance spectra to decrease at characteristic wavelengths. Starting from 400 nm, the spectra are determined mainly by scattering on the hydroxyapatite matrix [2, 32]. In enamel, tartar acts as a light absorber – hardened plaque, mineralized bacterial plaque (biofilm). It is formed on the surface of the teeth from food debris, dead microorganisms, calcium, phosphorus, and iron salts and determines the peak in the UV region of the enamel absorption spectrum.

The Kubelka-Munk reconstructed absorption spectra of dentin and enamel before and after tetracycline diffusion from aqueous solution are shown in Fig. 4.

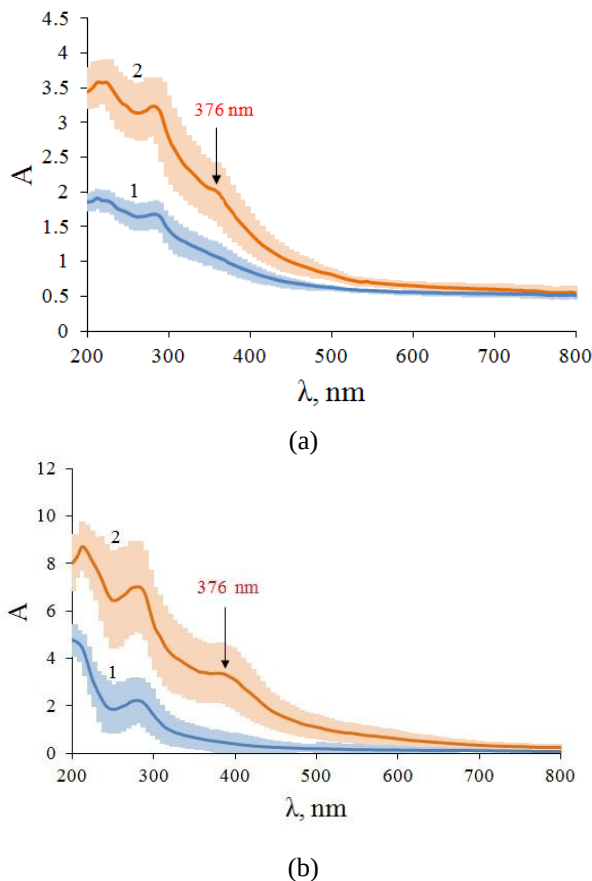


Fig. 4 Absorption spectra of dentin (a) and whole tooth with enamel (b) obtained from diffuse reflectance spectra reconstructed using the Kubelka-Munk algorithm: 1 – before tetracycline diffusion; 2 – after 5 h of tetracycline diffusion ($n = 3$).

In the process of diffusion of the agent, the spectra change with increasing the absorption in the UV region. After the penetration of tetracycline into dentin and enamel, the spectra show an increase in peaks at wavelengths characteristic for tetracycline. At 220 nm and 257 nm, the tetracycline bands coincide with the bands of the tooth endogenous chromophores; therefore, tetracycline identification in tissues was performed at 376 nm. After being in solution with the agent for 5 h, a peak is clearly detected in the absorption spectra at 376 nm, which indicates the presence of tetracycline in the samples (curve 2 in Fig. 4(a, b)).

The fluorescence spectra of the studied samples at an excitation wavelength of $\lambda = 376$ nm are shown in Fig. 5.

The original dentin fluorescence spectrum (Fig. 5(a)) has a peak at 460 nm with an intensity of up to 58 a.u., which is caused by endogenous chromophores with an absorption peak in the UV region. Tetracycline strongly absorbs in UV region and therefore partially does not transmit fluorescence at short wavelengths – self-absorption. The whole tooth with enamel fluorescence spectrum (Fig. 5(b)) has peaks at 413 nm, 437 nm, and 462 nm. Self-absorption in the whole tooth is greater than

for dentin (Fig. 5). Dentin samples are well impregnated with tetracycline through the dentinal tubules and transverse processes of these canals, when a whole tooth is examined, then diffusion into the enamel for 5 h is quite insignificant, therefore tetracycline works as a filter that attenuates fluorescence from all possible chromophores. We find a slight increase in fluorescence at 550 nm from 16 a.u. in the original enamel sample up to 20 a.u. in a sample with tetracycline.

Thus, we see that the penetration of tetracycline in fully mineralized adult teeth is significant in the dentin and less in the enamel, which is also well seen in the photographs of samples before and after staining, taken using a video densitometer (Fig. 6).

Fig. 6 shows samples of a human tooth, where various structural elements are indicated: 1 – enamel, 2 – dentin, 3 – cement. The original samples did not contain tetracycline in any of the regions (Fig. 6(a, b)). The photo of tooth sample (Fig. 6(c)) after being in the tetracycline aqueous solution for 5 h shows that the dentin was completely stained and began to have a yellowish tint, which is not visible on the enamel section.

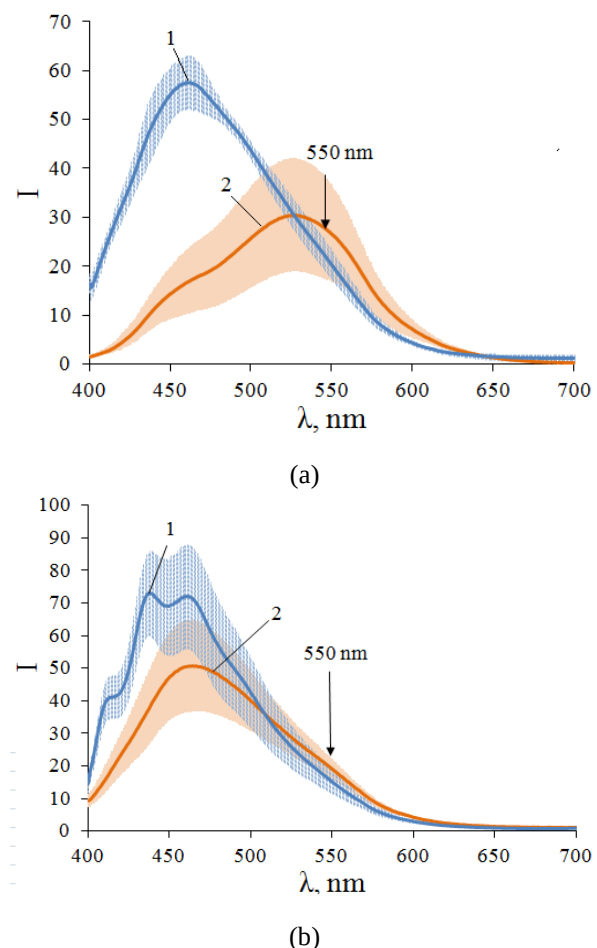


Fig. 5 Fluorescence spectra of dentin (a) and whole tooth with enamel (b): 1 – initial, 2 – after tetracycline diffusion from aqueous solution ($C = 1.1 \cdot 10^{-4}$ Mol/l) at excitation wavelength $\lambda = 376$ nm ($n = 3$).

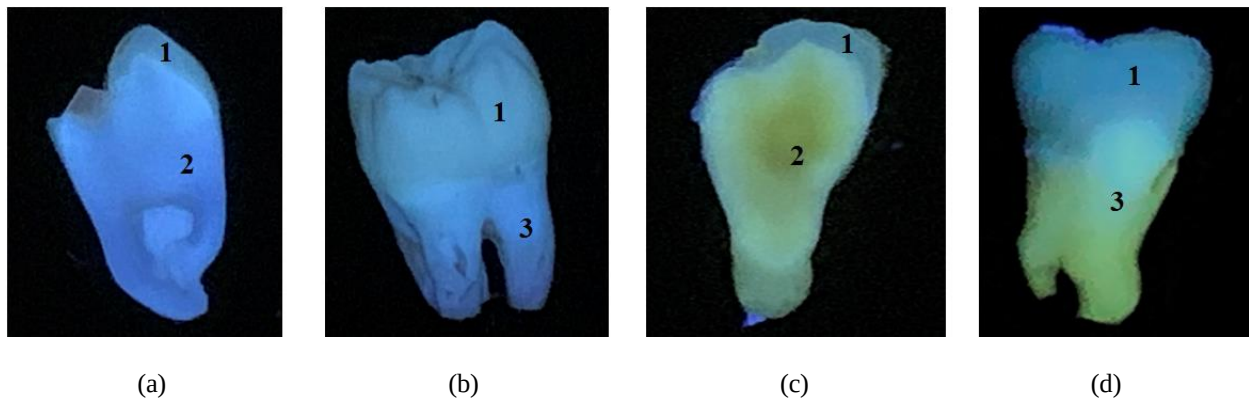


Fig. 6 Photographs of human tooth samples (1 – enamel, 2 – dentin, 3 – cement): (a) original tooth section, (b) original whole tooth, (c) – tooth section after tetracycline solution diffusion, and (d) whole tooth after tetracycline solution diffusion (emission spectrum at the 365 nm).

Table 1 Biophysical characteristics of human dentin samples.

Cut number	$D \times 10^6 \text{ cm}^2/\text{s}$	Thickness $l, \text{ cm}$	Average diameter of dentinal tubules, μm	Density of the number of dentinal tubules, $1/\text{mm}^2$
1	5.25	0.05	1.98 ± 0.34	10023
2	6.97	0.06	1.50 ± 0.41	6790
3	7.25	0.05	3.08 ± 0.94	27000

Fig. 6(d) shows a whole human tooth that has been in tetracycline solution for 5 h. It can be seen that the cement of the tooth was intensively stained with the agent, and the changes in the enamel are visually hardly noticeable.

Dentin has high permeability due to the presence of a huge number of tubules that penetrate it. This is of clinical significance, causing a rapid response of the pulp to damage of dentin. The ground substance surrounding the tubules is more compacted (hypermineralized) and homogeneous than the substance in the spaces between them. In this regard, peritubular (around the tubules, that forming their walls) and intertubular dentin are distinguished.

During caries (demineralization) peritubular dentin undergoes intense destruction, which leads to the expansion of the tubules and an increase in their permeability.

The location of collagen fibers and their structure change in different parts of dentin. In this regard, two layers of dentin are distinguished: outermost, or mantle dentin and innermost, or peripulpal (circumpulpal) dentin. Mantle dentin smoothly transitions into peripulpal dentin, which is less mineralized and contains larger dentinal tubules than mantle dentin [33, 34]. The mantle dentin matrix is more mineralized than the peripulpal dentin matrix and contains relatively fewer collagen fibers [34, 35].

Indeed, composition of dentin has impact on molecular diffusion. In the present study, we received averaged data for both types of dentin - mantle and peripulpal. However, proposed technology can be applicable for differentiated studies, which will need

specially prepared samples and monitoring of their structure using electron microscopy. The diameters of the dentinal tubules were $0.794 \mu\text{m}$ and $1.0 \mu\text{m}$ for the first and second molars, respectively (measurements were taken at a distance of 35–65% from the walls of the pulp chamber). The tubule density was $17,997.594 \text{ tubules}/\text{mm}^2$ and $25,211.317 \text{ tubules}/\text{mm}^2$ for the first and second molars, respectively [36].

Peripulpal dentin forms the majority of the dentinal layer and consists mainly of a layer of immature predentin with a constant thickness of 15–20 μm and a layer of dentin with increasing mineralization, starting from the mineralization front to the mantle dentino-enamel junction, and thickness of the order of several hundred micrometers, which significantly exceeds the thickness highly mineralized mantle dentin, which is 15–30 μm .

The studied samples included both zones of dentin, peripulpal, and mantle, because as it follows from Table 1 average diameter of dentinal tubules and their density was quite different. The average for 3 samples diameter of dentinal tubules is $2.2 \mu\text{m}$ and the density of the number of dentinal tubules is approximately 14600 per mm^2 . Data for each sample are shown in Table 1.

The mean value of diffusion coefficient of tetracycline was obtained as $D = (6.49 \pm 1.12) \cdot 10^{-6} \text{ cm}^2/\text{s}$ [28]. The measured properties of each three dentinal cuts are presented in Table 1. Let us consider dentin as a two-layer tissue of mantle and peripulpal layers, then total diffusion coefficient can be calculated as following [37]:

$$D = (l_1 + l_2)^2 / [(l_1)^2/D_1 + (l_2)^2/D_2], \quad (1)$$

where l_1 and l_2 are thicknesses of layers, D_1 and D_2 are diffusion coefficients in these layers, respectively.

Accounting that the peripulpal dentin matrix is less mineralized and denser than the mantle dentin matrix, let us assume that the sample with the highest diffusion rate was cut mostly from the peripulpal layer closer to pulp: $D_1 = 7.25 \cdot 10^{-6} \text{ cm}^2/\text{s}$. The high density of dentinal tubules 27000 per mm^2 and their relatively large diameter 3.08 ± 0.94 also indicate that the sample belongs to the inner layer of dentin. At the same time, samples 1 and 2 can be attributed to the outer layer of dentin, consisting of a more mineralized part of peripapillary dentin and mantle dentin. To be specific, let us take sample 1, which diffusion coefficient is $D_2 = 5.25 \cdot 10^{-6} \text{ cm}^2/\text{s}$. Then, using the Eq. (1), it is possible to estimate the average diffusion coefficient for dentin sample, consisting of different layers. Assuming that the thickness of the layers is the same, it is possible to calculate the average diffusion coefficient of dentin sample of 0.1 cm thick, which include both layers of dentin, internal and external, as $D_1 = 7.25 \cdot 10^{-6} \text{ cm}^2/\text{s}$ and $D_2 = 5.25 \cdot 10^{-6} \text{ cm}^2/\text{s}$ at $l_1 = l_2 = 0.05 \text{ cm}$. Thus $D = (l_1 + l_2)^2 / [(l_1)^2/D_1 + (l_2)^2/D_2] = 1.22 \cdot 10^{-5} \text{ cm}^2/\text{s}$.

4 Conclusions

In this study tetracycline penetration into the enamel and dentin of the tooth was studied by fluorescence

spectroscopy. Preliminary the absorption and fluorescence spectra of a solution of tetracycline hydrochloride were obtained. This agent has been shown to fluoresce with a peak at 550 nm at an excitation wavelength of 376 nm. It has been shown that tetracycline penetrates little through the enamel as it relatively well impregnates dentin. In *in vitro* conditions tetracycline comes from the pulp into the dentin and stains it, changing the color of the tooth. Therefore, it is important to study diffusion of tetracycline hydrochloride in dentin. The cement of the tooth is also stained significantly. In *in vitro* conditions tetracycline enters cement through the surrounding blood vessels and partially through the pulp. The quantitative determination of tetracycline in the structural components of the tooth requires further study.

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Disclosures

The authors declare that they have no conflict of interest.

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A New Discrimination Method of Imaging Regions for Improved Ultrafast Ultrasound Imaging Performance

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Abstract. Up to date, adaptive beamforming technologies have been successfully introduced to medical ultrasound imaging, resulting in a considerable improvement in imaging quality versus non-adaptive delay-and-sum beamformers. Minimum Variance (MV) adaptive beamforming improved resolution rather than contrast. At the same time, Eigen Space Based Minimum Variance (ESBMV) was formerly projected to enhance contrast in MV, but at the expense of the appearance of black regions around hyperechoic targets. Partial ESBMV (PESBMV) method has recently controlled the appearance of these black regions with a little reduction in the level of contrast. In this paper, a new technique of beamformer is proposed to improve the imaging quality of PESBMV. This approach uses two factors as a detection tool to adaptively indicate the regions of the image, then it applies the suitable beamforming method in each region. The results show that lateral resolution increased by 52% compared to that in PESBMV. Moreover, the contrast ratio is also increased with the preservation of the homogeneity of the background speckle. The proposed method is compared to MV, ESBMV, and PESBMV using *in vitro* experimental radio frequency data, showing improvement in the speckle preservation without affecting lateral resolution, and finally providing excellent image contrast performance. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: ultrasound imaging; plane wave imaging; minimum variance; eigenspace-based minimum variance; partial eigenspace-based minimum variance.

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1 Introduction

Ultrasonography has been applied for imaging the body of humans for more than 50 years. The Austrian neurologist was the first to use ultrasonography as a medical diagnostic tool to see the head [1, 2]. As a diagnostic instrument, imaging in ultrasound is one of the most common imaging technologies in the medical imaging field [2]. Medical ultrasound imaging has been introduced for a variety of applications over the last few decades, and its capabilities have been expanded beyond the traditional B-mode and Doppler functions, shear-wave elastography, and other advanced applications [3] as well as mapping of ultrafast blood flow [4]. It is portable, radiation-free, and very affordable in

comparison to other imaging technologies, like computed tomography and magnetic resonance. In addition, ultrasound images are tomographic, i.e., present a “cross-sectional” view of anatomical structure [2]. In the field of medical ultrasound, researchers and engineers searched to enhance image quality and frame rates [5, 6]. Plane wave imaging (PWI) is a form of imaging in which an unfocused single beam is utilized to visualize the imaging region. PWI creates a complete image with each transmission. Consequently, a high frame rate is incorporated in PWI composed of several thousand frames per second [7, 8]. Using the principle of PWI, which has been integrated into most sectors of medical ultrasonography, this field has undergone the revolution [6, 9]. Beamforming is the process of creating

an image from ultrasound echo signals generated from each array element [8]. This process is the most crucial step in revealing the information that passed reflected echo signals, otherwise, the ultrasound imaging procedure is meaningless [8]. Image quality in ultrasonography, specifically resolution, and contrast, is affected by the properties of the beam [10, 11]. The beamformer's main purpose is to generate a beam with reduced side lobes, on a depth, as well as a narrow main lobe [11, 12]. Regrettably, these are two contradictory objectives. The width of the main lobe affects the resolution of the image, while image contrast is determined by the sidelobe level and influences the dynamic range of image visualization [11].

The delay and sum (DAS) beamforming algorithm is the criterion technique for image reconstruction but with the limited imaging resolution and reduced off-axis interference rejection. The concept is to construct a picture by delaying the received signals from each aperture channel and summing resulting values. Weights are assigned to the received signals based on the receiving element's location, giving larger weights for central element signals, while elements located further out from the center are given lower weights [8]. By reducing the sidelobe level, this apodization operation improves imaging quality. The weight of apodization is constant and independent on data and has the disadvantage of broadening the mainlobe and decreasing lateral resolution [8]. Therefore, adaptive beamforming was developed. The minimum variance (MV) beamformer is an adaptive beamformer, which Capon pioneered in 1969. It is so named because it minimizes the output power, which is used to calculate the weighting vector while conserving the focal point response. Over the years, several strategies for enhancing MV beamformers have been presented. Mann proposes the frost beamformer in 2002 [13]. Sasso and Cohen-Bacrie proposed the spatial smoothing technique for decorrelating signals on and off the axis, as well as a well-conditioned covariance matrix [14]. Synnevåg et al. used diagonal loading in estimating the covariance matrix [15]. In addition, they suggested a temporal averaged MV beamformer, where temporal averaging and spatial smoothing were utilized to preserve speckle statistics [15]. In MV, resolution is significantly improved compared to DAS, but contrast is still unsatisfactory. Here is where Eigenspace-based methods are introduced. The working assumption is to divide the covariance matrix into two orthogonal subspaces, signal and noise. The signal subspace describes the mainlobe signals information, while the noise subspace matches the sidelobe and off-axis signals [16, 17]. Asl and Mahloojifar presented eigenspace-based minimum variance (ESBMV) beamformer approach, which tells that the beamformer weights are obtained by projecting the MV weights onto the generated signal subspace [17, 18]. With using this method, the contrast is significantly improved, at the expense of presenting artificial dark spots called black-box regions (BBRs) at the sides of hyperechoic targets and the background

speckle. In 2017, the partial-ESBMV (PESBMV) method was proposed to overcome the limitation of ESBMV. This method states that according to the signal subspace's eigenvector number, ESBMV is applied or blocked. This method thus managed to reduce BBR artifacts [8], but the contrast is decreased compared to ESBMV. After that, several methods were suggested to improve ESBMV's performance such as short-lag spatial coherence combined with ESBMV proposed to remove BBR artifacts under a high threshold of eigenvalues [19]. Also, ESBMV has been combined with Delay Multiply-And-Sum (DMAS) beamformer to decrease sidelobes level and increase the signal-to-noise ratio, this could not remove ESBMV's artifact [20]. It was also suggested to combine ESBMV with the sign coherence factor to improve the signal-to-noise ratio, but dark spots and BBRs still exist [21].

This paper presents a new method which is an upgraded version of the PESBMV. This method divides the imaging region into four areas based on their properties that directly affect the weight produced by ESBMV, in addition to the number of eigenvectors in the signal subspace matrix in ESBMV. This discrimination helps to use the beamforming technique that suits each region, resulting in improved overall ultrasound image quality.

2 Background Methods

2.1 Minimum Variance Beamformer (MV)

The weighting vector in an MV beamformer is always reorganized to minimize the output power through keeping unity gain for the response from the focal point, this requirement can be mathematically explained as [14, 15, 22]:

$$\mathbf{w}_{MV} = \arg \min_{\mathbf{w}} \mathbf{w}^H \mathbf{R} \mathbf{w} \text{ subject to } \mathbf{w}^H \mathbf{a} = 1, (1)$$

where the weighting vector is $\mathbf{w}$, $\mathbf{R}$ is the echo data's covariance matrix, $\mathbf{a}$ is defined as the steering vector accustomed to recompense for the delays from the focal point for each receiving element. The result is presented as [15, 22]:

$$\mathbf{w}_{MV} = \frac{\mathbf{R}^{-1} \mathbf{a}}{\mathbf{a}^H \mathbf{R}^{-1} \mathbf{a}}. (2)$$

The analytical form of $\mathbf{R}$ is unknown and is typically estimated from the data. In spatial smoothing where the element transducer array is divided into $\mathbf{P}$ subarrays, every subarray is identical to its neighboring subarray, however with one element shifted [23] Temporal smoothing, where $(2K + 1)$ is a vector of length-selected samples for each focal point [24] is included in the $\mathbf{R}$ calculation. The covariance matrix can be expressed as [15, 22]:

$$\mathbf{R} = \frac{1}{P} \sum_{p=0}^{P-1} \mathbf{G}_p \mathbf{G}_p^H, (3)$$

where the number of subarrays is p , and it equals $(M - L_p + 1)$, where L_p is the length of the subarray in the spatial smoothing operation and M is the overall number of transducer elements. G_p is the p^{th} subarray, as follows [15, 22]:

$$G_p = [y_p(n) \quad y_{p+1}(n) \quad \dots \quad y_{p+L_p-1}(n)]', \quad (4)$$

where $y_p(n)$ is a segment of the received input signal p^{th} element, $p = 0, 1, \dots, P - 1$. $y_p(n)$ represents a vector of length $(2K + 1)$. The beamformer's output value is computed by multiplying the subarray average by the weighting vector [15, 22]:

$$Y = w_{MV}' \frac{1}{P} \sum_{p=0}^{P-1} G_p. \quad (5)$$

By studying the change of the value of L_p (Spatial smoothing technique with subarray length) on the contrast and resolution in MV and ESBMV methods, it is observed that a decreasing in the value of L_p leads to the lower contrast ratio, and contrast to noise ratio (approach to DAS beamforming method), and also decreases resolution and increases brightness, while the background speckle appears homogeneous and clear, one the other side the increase in the L_p value has a very positive and noticeable effect on the resolution and contrast but at the expense of reducing homogeneity of background speckle.

2.2 Eigen Space Based Minimum Variance (ESBMV)

The MV covariance matrix ($\mathbf{R}$) is separated into signal and noise subspaces, with a weighting vector projected to signal subspace. $\mathbf{R}$ can be written as following [27, 28]:

$$\mathbf{R} = \mathbf{V}\mathbf{A}\mathbf{V}^H = \sum_{i=1}^{L_p} \lambda_i \mathbf{v}_i \mathbf{v}_i^H, \quad (6)$$

where the diagonal matrix is $\mathbf{A} = \text{diag}[\lambda_1, \lambda_2, \dots, \lambda_{L_p}]$, $\mathbf{R}$'s eigenvalues are represented by the diagonal, where $\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_{L_p}$ are arranged in decreasing order. $\mathbf{V} = [\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_{L_p}]$ where $\mathbf{v}_i$ is the i^{th} orthonormal eigenvector for λ_i with $i = 1, \dots, L_p$. In this method, $\mathbf{R}$ is divided based on its Eigen structure, into signal and noise subspaces. Subsequently, the MV weighting vector is planned onto the corresponding subspace-constructed signal. As a result of the high coherency of on-axis signals, the energy produced by the mainlobe is focused on the eigenvectors related to the larger eigenvalue. Thus, the signal subspace matrix ($\mathbf{E}_s$) is written as following [18]:

$$\mathbf{E}_s = [\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_{Num}], \quad (7)$$

where Num is the number of eigenvectors included in the signal subspace. The signal subspace is constructed from the eigenvectors whose corresponding eigenvalues

are greater than δ times the highest eigenvalue (λ_{max}), where δ is a value of 0 to 1 and is set by the user [29]. New weights are then calculated by projecting the MV weight to the signal subspace using the following Eq. [18]:

$$\mathbf{w}_{ESBMV} = \mathbf{E}_s \mathbf{E}_s^H \mathbf{w}_{MV}. \quad (8)$$

2.3 Partial Eigenspace-Based Minimum Variance (PESBMV)

Contrast and resolution in MV adaptive beamformers are improved by ESBMV beamforming. Nevertheless, two types of artifacts limit the execution of ESBMV. Firstly, BBR around hyperechoic targets, and secondly dark spots in background speckles. Therefore, PESBMV was proposed to overcome those limitations. By depending on the value of Num , this method can distinguish or divide the image into two areas. The first area contains hyperechoic objects, wires, and sidelobe and, the second area contains hypoechoic objects and speckle backgrounds. The weight in this method can be written as following [8]:

$$\mathbf{w}_{PESBMV} = \begin{cases} \mathbf{E}_s \mathbf{E}_s^H \mathbf{w}_{MV} & \text{if } Num > L_p \cdot \eta \\ \mathbf{w}_{MV} & \text{otherwise.} \end{cases}, \quad (9)$$

where η is a user-specified coefficient that varies between 0 and 1. PESBMV was able to achieve a quantum leap in the PWI field by averting the BBR artifacts and controlling the dark spots through changing the value of η .

2.4 Coherence Factor (CF)

The coherence factor (CF) method is frequently used to describe the superiority of adaptive imaging focusing [26]. CF is defined as following [27, 28]:

$$CF(k) = \frac{|\sum_{l=1}^L x_l(k)|^2}{M \sum_{l=1}^L |x_l(k)|^2}, \quad (10)$$

where $x_l(k)$ represents the data received from channel l after applying focusing delay and k is the time index. In ultrasonic imaging, CF value is commonly utilized as a weight to curb sidelobes. The ultimate beamformed output is found by following [26]:

$$y_{CF} = CF \cdot Y. \quad (11)$$

2.5 Proposed Methods

The new method is proposed to maintain high imaging quality. It depends on the value of Num and the weight of ESBMV to distinguish between the regions. This method is proposed to take advantage of the properties of ESBMV in terms of contrast and MV in terms of reversing the homogeneous background speckle.

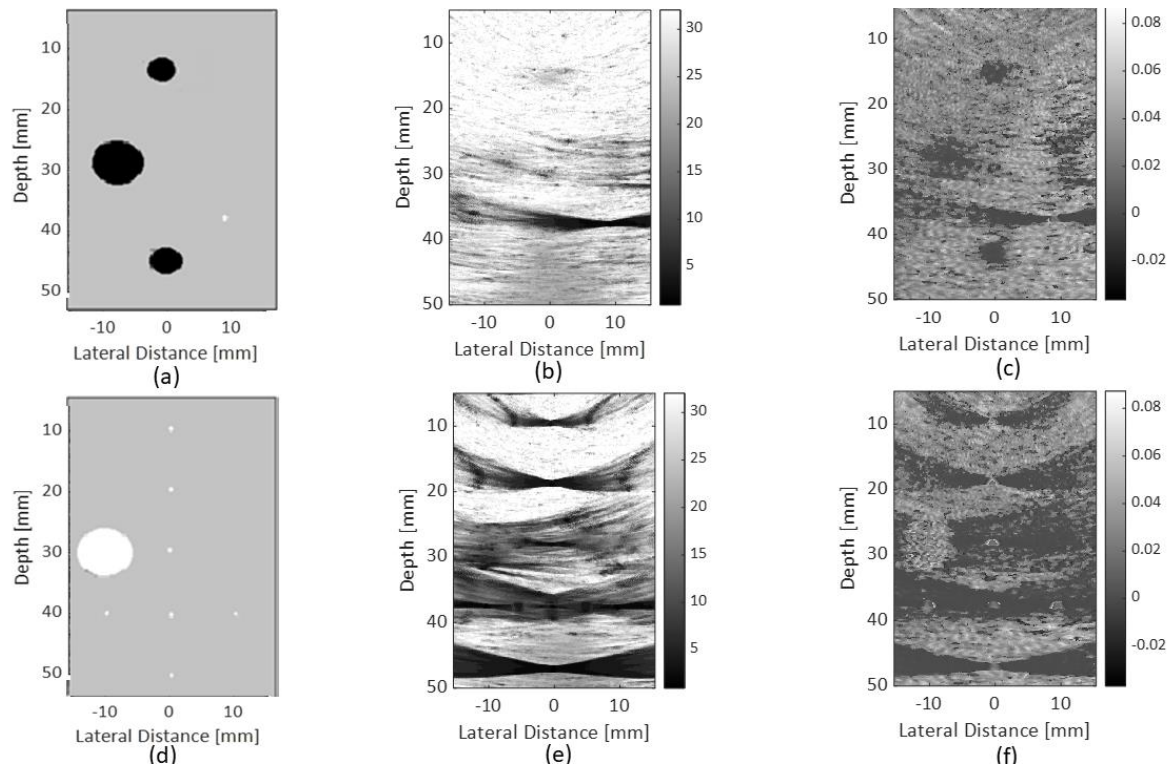


Fig. 1 The value of Num , using reference ESBMV at $\delta = 0.2$, $Lp = 32$ in panel (b) for contrast phantom shown in panel (a) and panel (e) for resolution phantom shown in panel (d). Panel (a) shows a contrast phantom with three cysts centered at the 15 mm and 42 mm depths; panel (d) shows a resolution phantom with 7 wires hyperechoic lesion centered at the 26 mm depth. Panels (c) and (f) show the weight of ESBMV at $\delta = 0.2$, $Lp = 32$ using contrast and resolution phantoms, respectively.

The idea is to exploit the positives of all the previously used methods and depending on specific values that discriminate and detect different areas of the image, the best performance of the methods is included in each region to obtain a high-quality image.

In this paper, two factors are proposed to distinguish between the different areas of the image. The first parameter is the value of Num by which the image is divided into two parts, the first is hyperechoic targets and sidelobe regions, and the second part is the hypoechoic target and background speckle. Afterwards, each of these two regions is further discriminated depending on the weight produced by ESBMV and on Num . The behavior of those two discrimination factors is emphasized in Fig. 1.

After discrimination, the proposed method uses ESBMV ($Lp = M/2$) multiplied by CF for hypoechoic target, and MV ($Lp = 1$) for background speckle and hyperechoic target regions.

Finally, MV ($Lp = M/2$) is used for areas that include BBRs. The flow chart that explains the steps followed by this method is shown in Fig. 2.

The hyperechoic target is distinguished by observing the Num value, which is equal to one, as shown in Fig. 1. Also, the differentiation between the hypoechoic and background speckle was performed through the observation of the normalized ESBMV weight at ($Lp = M/4$), which ranges between zero to 0.5 at

hypoechoic targets, and between 0.5 to one at the background speckle.

3 Implementation and Experimental Datasets

3.1 Experimental Datasets

Ultrasound imaging quality is highly affected by the imaging parameters and settings, such as the type and width of the transducer, central frequency, bandwidth, etc. [29]. Therefore, the proposed beamforming method is tested and compared to standard beamformers under fixed imaging settings.

Two experimental datasets are used in this work. Those datasets were obtained from the web platform of the International Ultrasound Symposium IEEE 2016 held in Tours (France) [30]. A Verasonics Vantage 256 research scanner and an L11 probe were used to collect data (Verasonics Inc., Redmond, WA). CIRS Multi-Purpose Ultrasound Phantom (Model 040GSE) was used to collect the datasets as shown in Figs. 1(a) and (d) [31]. The first dataset is shown in Fig. 1(a) and has three anechoic cysts against a background speckle. The second dataset is shown in Fig. 1(d) [6]. The Matlab program is used to implement the proposed method as well as MV , ESBMV, and PESBV methods for assessment and compression. Table 1 gives the detailed settings used through data acquisition.

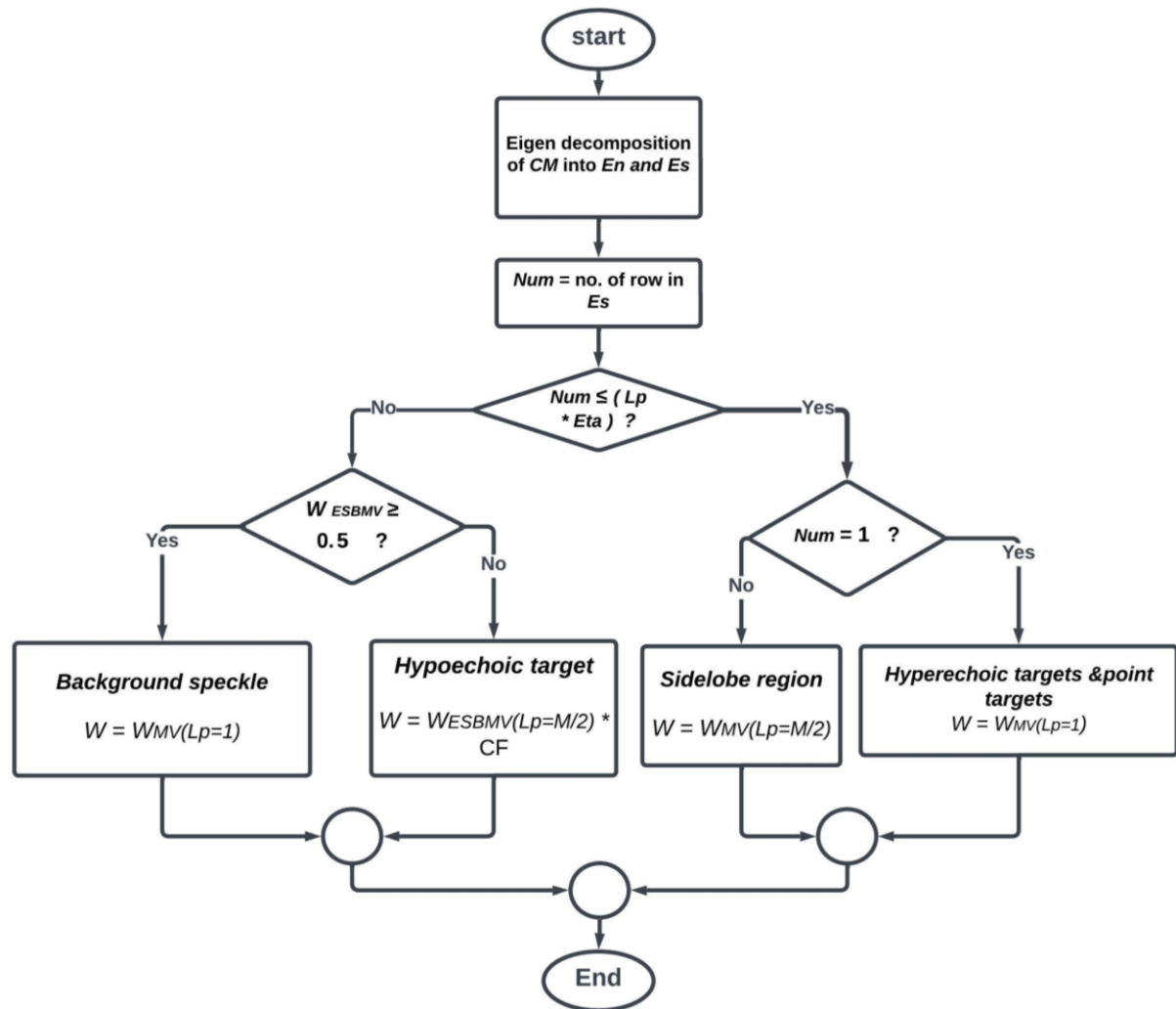


Fig. 2 Flow chart outlining the steps followed by the proposed method.

Table 1 The imaging settings employed during data acquisition.

Pitch	0.30 mm
Element width	0.27 mm
Element height	5 mm
Elevation focus	20 mm
Number of elements	128
Aperture width	38.4 mm
Transmit frequency	5.208 MHz
Sampling frequency	20.832 MHz
Pulse bandwidth	67%
Excitation	Cycles

3.2 Quality Metrics

In this section, well-known qualities for quantitative analyses of beamforming performance was used. Those metrics are contrast ratio (CR), contrast-to-noise ratio

(CNR), speckle-to-noise ratio (SSNR), and full width at half maximum (FWHM).

CR indicated in the following equation. CR is used to evaluate imaging contrast; CR is determined from the absolute difference between the cystic area's mean value and the background tissue [32]:

$$CR = |\mu_i - \mu_b|, \quad (12)$$

where the mean values within the cyst target and speckle are μ_i and μ_b , respectively.

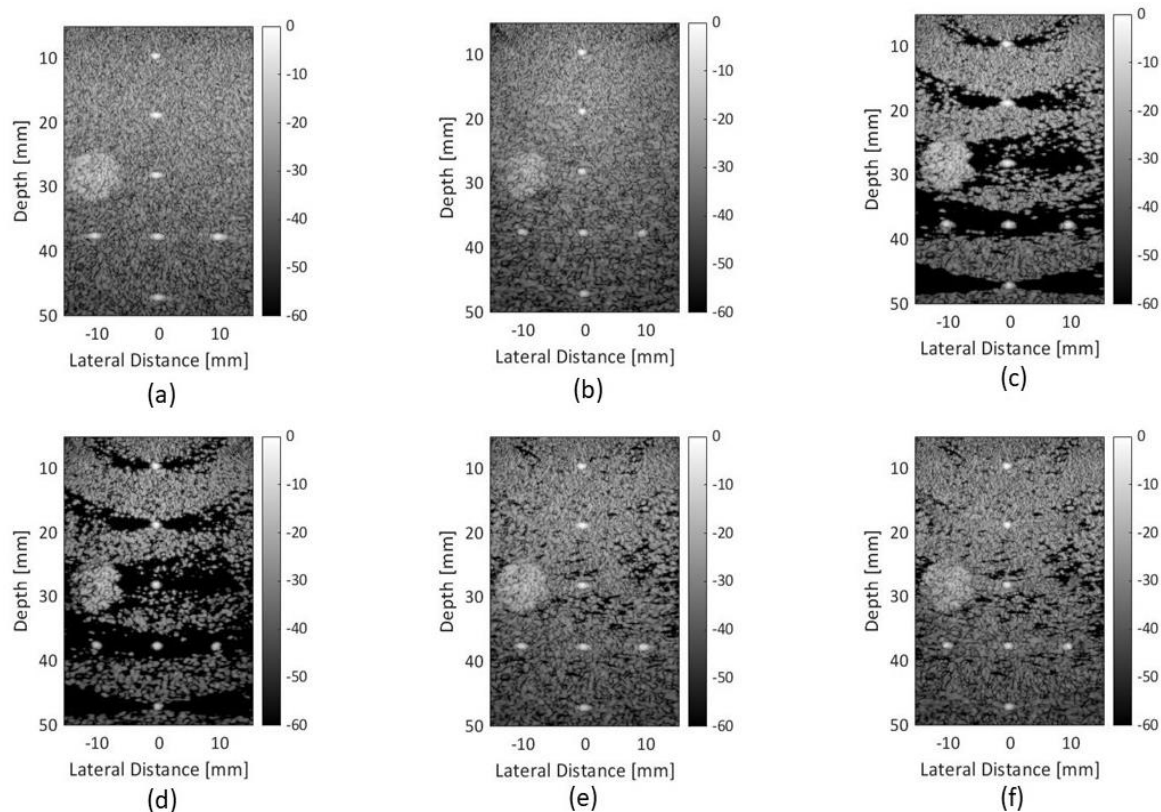
CNR is another measure of contrast that is found as follows [32]:

$$CNR = \frac{|\mu_i - \mu_b|}{\sqrt{\sigma_i^2 + \sigma_b^2}}, \quad (13)$$

where σ_i and σ_b are the equivalent standard deviations, within the cyst target and the speckle background, respectively. Two 2×1 squares inside cysts with two 11×15 mm squares to the left and right above corner of the image are the background areas against which CR and CNR are calculated.

Table 2 Measurements of contrast and speckle statistics for the contrast phantom using different beamforming techniques with PWI.

Method	FWHM (mm)	CR (dB)	CNR (dB)	SSNR
MV ($L_p = 1$)	0.87	12.36	2.05	1.74
MV ($L_p = M/2$)	1	11.85	1.97	1.65
ESBMV ($L_p = M/4$)	0.77	15.67	2.44	1.63
ESBMV ($L_p = M/2$)	0.9	17.17	2.12	1.30
PESBMV ($\delta = 0.2, \eta = 0.5$)	1.1	15.57	2.42	1.63
Proposed method ($\delta = 0.2, \eta = 0.5$)	0.57	16.26	2.55	1.7

Fig. 3 Images of the experimental RF data of the resolution phantom using: (a) MV ($L_p = 1$), (b) MV ($L_p = M/2$), (c) ESBMV ($\delta = 0.2, L_p = M/4$), (d) ESBMV ($\delta = 0.2, L_p = M/2$), (e) PESBMV ($\delta = 0.2, \eta = 0.5, L_p = M/4$), and (f) proposed method ($\delta = 0.2, \eta = 0.5$). All images are shown in a dynamic range of 60 dB.

Speckle signal to noise ratio ($SSNR_{Bg}$) is used to evaluate the quality of speckles. The following Eq. is used to determine $SSNR_{Bg}$ [33, 34]:

$$SSNR_{Bg} = \frac{\mu_b}{\sigma_b}, \quad (14)$$

In contrast phantom, 11×17 mm square in the right top corner is used to measure the background speckle's homogeneity using $SSNR_{Bg}$. As shown in Eq. (15), a similar formula is used to calculate the BBR's speckle-signal-to-noise-ratio ($SSNR_{BBR}$) [33, 34]:

$$SSNR_{BBR} = \frac{\mu_{BBR}}{\sigma_{BBR}}, \quad (15)$$

where μ_{BBR} and σ_{BBR} are the BBR's mean and standard deviation values. In the resolution phantom, two 2×2 mm to the sides of the wire target at a depth of 9 mm indicates the area for which, BBR artifacts are measured using $SSNR_{BBR}$.

Calculating the FWHM (6-dB beamwidth) of the main lobe in the lateral direction gives the lateral resolution [35].

4 Results and Discussion

In this work, a variety of beamformers is applied to the used datasets to evaluate the proposed beamformer's performance. Fig. 4 displays the superiority of the

proposed method in improved CR, where, as shown in Fig. 4(a) and (b) MV ($Lp = 1$), MV ($Lp = M/2$) respectively produce blurred boundaries for the anechoic cyst target. While there is visible noise in ESBMV ($Lp = M/4$), ESBMV ($Lp = M/2$), and PESBMV ($Lp = M/4$) decrease CR compared to ESBMV ($Lp = M/4$) as shown in Table 2.

The speckle pattern produced by MV ($Lp = M/2$) as shown in Fig. 4(b) is sandy and inhomogeneous, with significantly darker speckle intensity, while in the ESBMV method, the background speckle suffers from BBR. The PESBMV method is better than ESBMV in limiting dark spots and BBR. Nevertheless, the background speckle is very homogeneous using MV ($Lp = 1$ and $M/2$) as in Fig. 4(a) and (b). This is comparable to DAS. Table 2 indicates that SSNR for the proposed method and MV ($Lp = 1$) is close, this illustrates the strength of proposed method in preserving the homogeneity of speckle background.

Lateral responses of the implemented beamformers for the point target positioned at the 18.75 mm depth in the resolution phantom dataset are given in Fig. 5 and Fig. 3 to aid intuitive observations of their lateral resolution performance. The graphs show that when Lp increases, all MV-based methods considerably assist in reducing main lobe widths, where, compared to the other approaches, MV ($Lp = 1$) is less effective in improving lateral resolution since its main lobe width is similar to that of DAS. Fig. 5 shows that the worst resolution was achieved by ESBMV ($Lp = M/4$) but improved when increase Lp , as well as MV, lateral

resolution in PESBMV approach from ESBMV method, from Table 2 it can be noticed that the smallest value of FWHM has been achieved using proposed method, with an improvement by (52%) compared the resolution achieved using PESBMV.

The presented algorithm is shown to be highly effective in increasing the quality of imaging by improving image contrast and speckle pattern preservation, as well as significantly increasing lateral resolution. This is done by sensing the details of the image first through the value of Num , which can efficiently distinguish hyperechoic targets and sidelobe regions from the rest of the regions, as it gives a value (1) for the hyperechoic target and greater than one to BBR as shown in Figs. 1(b) and (e), and secondly, using the value of the weights of ESBMV ($Lp = M/4$). This is because the values of this weight given to the cyst range from (0–0.5), as can be noticed in Figs. 1(c) and (f).

Fig. 4 illustrates that the cyst is much more visible using proposed method and that the proposed beamformer significantly improves CNR, exceeding all the implemented beamforming methods. Fig. 4 illustrates that the cyst is much more visible using proposed method and that the proposed beamformer significantly improves CNR, exceeding all the implemented beamforming methods. The main reason for that is the ability of the proposed method to detect the areas of the cysts. Second, the use of the CF justifies the weight of beamformer based on the incoherency of these signals.

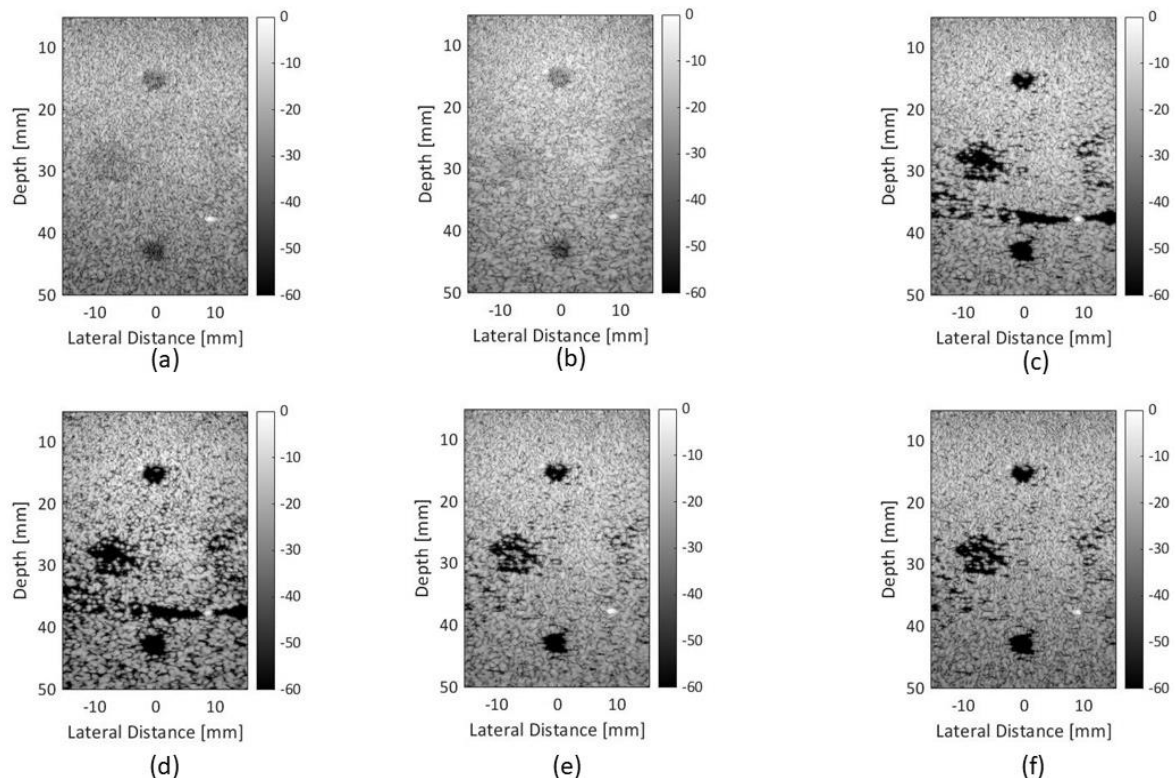


Fig. 4 Images of the experimental RF-data of cyst phantom using: (a) MV ($Lp = 1$), (b) MV ($Lp = M/2$), (c) ESBMV ($\delta = 0.2$, $Lp = M/4$), (d) ESBMV ($\delta = 0.2$, $Lp = M/2$), (e) PESBMV ($\delta = 0.2$, $\eta = 0.5$, $Lp = M/4$), and (f) proposed method ($\delta = 0.2$, $\eta = 0.5$). All images are shown in a dynamic range of 60 dB.

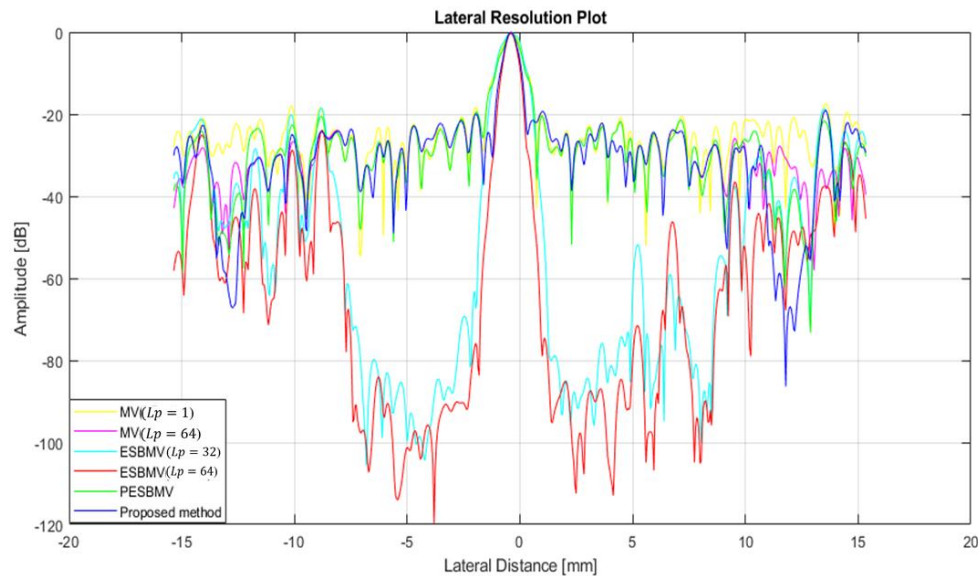


Fig. 5 FWHM displayed for the wire located at ($z = 18.75$ mm) depth of the dataset from resolution phantom.

Table 3 Measurements of black-box region and resolution for the resolution phantom using different beamforming techniques with PWI.

Method	FWHM (mm)	SSNR _{BBR}
MV ($Lp = 1$)	1.086	5.82
MV ($Lp = M/2$)	0.709	6.33
ESBMV ($Lp = M/4$)	1.129	2.29
ESBMV ($Lp = M/2$)	0.648	2.56
PESBMV ($(\delta = 0.2, \eta = 0.5)$)	0.987	6.03
Proposed method ($\delta = 0.2, \eta = 0.5$)	0.704	5.89

Third reason is the projection of the MV weights onto the signal subspace also. Also, to improve speckle statistics and a contrast in tissue areas, temporal smoothing is applied, where a vector of samples instead of a single sample is used to produce the final value of the focal point.

The results presented in Table 2 confirm the superiority of the proposed method in terms of CR and CNR. When the weight of ESBMV ($Lp = M/4$) are higher than (0.5), this indicates inside the areas of the speckle. The proposed method uses MV ($Lp = 1$) because through it more homogenous background speckle and higher values of SSNR are achievable.

The issue of underestimation of the amplitude of hyperechoic targets when a large length of subarray is used has been solved by surrounding the hyperechoic and wire areas by MV ($Lp = M/2$) while giving the hyperechoic and wire targets weights of MV ($Lp = 1$). The results of this method were obtained as described in Table 2 and Table 3.

5 Conclusions

In this paper, we introduce a novel approach for improving image quality. The new algorithm which

discriminates imaging area based on Num and the weight of ESBMV is meant to improve lateral resolution and speckle preservation while simultaneously increasing contrast by using different beamforming types in each defined area. Experimental results show that the proposed approaches can achieve increased image contrast and very well keep speckle patterns with a significant increase in lateral resolution. Additionally, the proposed approach has the potential to be an effective strategy for improving image quality in other imaging methods as well as Compound Plane Wave Imaging (CPWI).

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Disclosures

The authors declare no conflict of interest.

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Optimization of Preliminary Vector Averaging for Improving Strain-Estimation Accuracy in Phase-Sensitive Optical Coherence Elastography

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Abstract. In this paper we present a method that significantly improves strain estimation quality in phase-sensitive optical coherence elastography (OCE), in which interframe strains are proportional to gradients of interframe phase variations. The analyzed phase-sensitive OCT signals can be conveniently represented as complex-valued vectors, the arguments of which correspond to the sought interframe phase-variations. To reduce the noise influence on the strain-estimation accuracy, these complex-valued vectors are usually averaged over a certain window. Increase in the window size can greatly reduce the noise. At the same time, for inappropriately big windows, this smoothing may cause strong distortion of the reconstructed spatial distribution of strain in comparison with the actual distribution, especially in regions of large gradients of interframe phase variations and, correspondingly, increased strain level. Therefore, automatic adaptive choice of the window size is proposed to maximize the noise reduction and at the same time to avoid possible distortion of spatial strain distribution in the reconstructed strain maps. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: optical coherence elastography; OCE; automatic choice of parameters; adaptive averaging.

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1 Introduction

In recent years, besides straightforward perfection of obtaining structural images in optical coherence tomography (OCT), the further progress is strongly focused on the development of new OCT modalities/extensions [1]. Their realization requires deep analysis of series of OCT scans, for example, for realization of OCT-based angiography [2] and elastographic modality in OCT [3, 4]. In OCT-based elastography, starting from the first studies [5], special attention is paid not only to characterization of elasticity of biological tissues, but also in a broader sense the problems related to visualization of displacements and strains have been actively studied. In this context, especially in earlier works, as well as in some more recent studies (e.g., [6–9]), utilization of correlation-based methods of speckle tracking was considered as was

initially proposed in Ref. [5] by analogy with ultrasound elastography [10] and strain mapping in mechanical engineering [11]. In the recent decade significant attention is paid to the development of strain-estimation methods based on phase-resolved OCT [4, 12–15]. In contrast to correlation-based tracking, in phase-resolved approaches the scatterer displacements are conveniently visualized without the necessity of search operations by straightforward evaluation of interframe phase variations. Then spatially-resolved strains can be found by numerically estimating the phase-variation gradients.

For enhancing the quality of such OCT-based strain estimation, various improvements of finding interframe phase-variations and their gradients were proposed. For example, least square fitting of the phase-variation depth dependence combined with amplitude weighting was considered in Ref. [12] to reduce contributions of the most noisy small-amplitude pixels. In studies [16, 17],

optimization of time intervals between compared OCT scans was considered for estimation of slowly-varying interframe strains and finding the cumulative strains (for example, such slow-strain visualization is required for estimating deformations caused by insufficiently relaxed internal stresses when controlling the shape of prepared implants [18], for studying osmotic deformations [19] or polymerization processes [17]).

For estimation of “instantaneous” interframe strains, the vector approach proposed in Ref. [13, 14] has become widely used in recent years by various groups [20–22]. In this approach, phase-resolved OCT signals are represented as complex-valued vectors in the complex plane. The vector approach is very efficient computationally [4, 22] and opens convenient possibilities for various averaging and weighting procedures, parameters of which should be optimized taking into account particular features of the reconstructed strain distribution. Since the spatial structure of strains and resultant interframe phase variations may strongly vary during the acquisition of OCT-signals, as well as may be strongly inhomogeneous within the field of view, the optimal parameters of such averaging/weighting procedures should be correspondingly adapted.

In phase-sensitive OCE, interframe displacements of scatterers determine interframe phase variations, so that to find interframe strains one should estimate gradients of interframe phase variations. This estimation may be performed, e.g. by applying least-square procedures to the depth-dependence of interframe phase variations [12] or using the above-mentioned vector approach [13, 14]. For the vector method of strain estimation, recent study [23] considered optimization of such an important parameter as the choice of scale (or step) over which gradients of interframe phase variations are estimated. In the presence of measurement noises, the analyzed phase-variation distribution is characterized by random fluctuations, so that the choice of a too small step when estimating gradient (the minimal step is evidently equal to one pixel) may cause very strong errors in the gradient and strain estimation. Choosing a larger scale helps to reduce errors in the phase-gradient estimation, but too large gradient-estimation steps may also degrade the estimated strain if the chosen step exceeds the spatial interval, beyond which phase-wrapping occurs for the current level of local strain. Such multiple wrapping of interframe phase variations within an OCT scan is quite a typical situation for rather moderate strains $\sim 10^{-3} \dots 10^{-2}$, for which the compared OCT scans are not yet strongly decorrelated and the interframe phase variations are well visible. In view of this, in work [23] a method was proposed for automatic choice of the differentiation scale corresponding to the local strain value. The main idea of the method is that this differentiation scale should be made so large that the estimated phase difference over the chosen scale approaches the maximal unambiguous value $\pm\pi$ rad (in other words, phase variation is close to wrapping over this spatial scale).

In the current paper we consider another important issue which also has to be optimized when finding displacement and strains using noisy phase-sensitive OCT signals. Namely, we will consider the problem of spatial averaging of interframe phase-variation maps to reduce noise level in the estimated strain. Generally speaking, the usefulness of averaging procedures for noise suppression in phase-sensitive OCT is fairly evident and in the vector method of strain estimation [14, 15] the averaging of complex-valued signals (also called phasors [24]) has been routinely used, but without adaptive optimization. However, similarly to the inappropriately large gradient-estimation scale, the useful signal may experience decrease in the resultant post-averaging amplitude because of phase-variation nonuniformity within a too large averaging window. On the contrary, for too small windows, the averaging does not yet appreciably help to reduce noises. Thus, similarly to the adaptive choice of gradient-estimation scale discussed in Ref. [23] for strain estimation using phase-sensitive OCT signals, the size of averaging windows should also be adaptively chosen taking into account the spatial distribution of reconstructed strains.

It is important that in the problem of strain estimation, the averaging can be used at various stages of OCT-scan analysis. Namely, the averaging (which in what follows is called “preliminary averaging”) can be applied to the initial results of pixel-to-pixel comparison of phase-sensitive OCT data. When displacements of scatterers are spatially inhomogeneous (i.e., different from simple translational displacements) the corresponding interframe phase variations are also spatially inhomogeneous. Consequently, even if the sought strain is homogeneous, the interframe phase-variation is spatially inhomogeneous and for too large averaging windows the summed signals may become essentially out-of-phase. This effect is the main factor that limits the size of preliminary-averaging window.

Additional averaging procedures (which will be called “final averaging”) can be applied at the following stages to the quantities containing phase-variation gradients proportional to the sought strains. These gradients usually exhibit much slower spatial variability and the window size for final averaging may be chosen significantly greater.

In the following sections we specifically consider the problem of adaptive automatic choice of the averaging-window size at the stage of preliminary averaging. Using both numerically simulated OCT scans and real OCT records we will demonstrate that such averaging may be rather efficient. Along with the adaptive choice of the phase-gradient-estimation scale, the adaptive choice of window size for preliminary averaging can be used to significantly improve the quality of reconstructed strain maps in phase-sensitive OCT. The result based on numerical simulations and processing of experimental OCT images will be complemented with analytical arguments elucidating the physical background of the considered averaging procedures.

2 Illustration How the Size of Preliminary-Averaging Window Affects the Quality of Interframe Phase Difference Estimation

Currently, the phase approach is most widely used in optical coherence elastography (OCE) when estimating displacements and strains [4]. An important advantage of the phase approach is that, unlike correlation methods, the axial displacement component field u can be found without the need for any search operations. The pixel amplitudes of phase-sensitive scans can be represented in the complex-valued form $a(m, j) = A(m, j)\exp[i\varphi(m, j)]$, where absolute amplitude $A(m, j) = |a(m, j)|$ and $\varphi(m, j)$ is the phase. Axial displacements $u(m, j)$ of scatterers lead to interframe variations in the phases $\Phi(m, j) = \varphi_2(m, j) - \varphi_1(m, j)$, which can be directly found by pixel-to-pixel comparison of the two subsequent OCT images, such that

$$\Phi(m, j) = \arg[a_2(m, j)a_1^*(m, j)], \quad (1)$$

where the asterisk denotes complex conjugation.

Thus, displacement $u(m, j)$ in every pixel with the axial index m and lateral index j can be expressed via these phase variations as [12]:

$$u(m, j) = \frac{\lambda_0 \Phi(m, j)}{4\pi n}, \quad (2)$$

where λ_0 is the central wavelength of the OCT source and n is the refractive index.

It is assumed that the interframe displacements may be of supra-wavelength magnitude producing phase wrapping, but they should not be essentially supra-pixel (otherwise, the pixel-to-pixel comparison makes no sense). In real conditions, the acquired complex-valued amplitudes $a_{1,2}(m, j)$ are characterized by some measurement errors. Consequently, the complex-valued product $a_2(m, j)a_1^*(m, j)$ and phase-variation field $\Phi(m, j)$ found using Eq. (2) are also noisy.

The amplitudes and phases of measurement noises (e.g., the reception-array noises of the OCT device) are independent for neighboring pixels, whereas the sought more regular interframe signal variations in contrast to measurement noises are slower varying over the OCT scan. Consequently, for suppression of noise level, the complex-valued scans $a_2(m, j)a_1^*(m, j)$ can be averaged over a window W_z by W_x pixels in size, where z and x correspond to the axial and lateral directions, respectively. Since quantity $a_2(m, j)a_1^*(m, j)$ represents a complex-valued vector in the complex plane, such averaging procedure can reasonably be called “vector averaging” [13, 14].

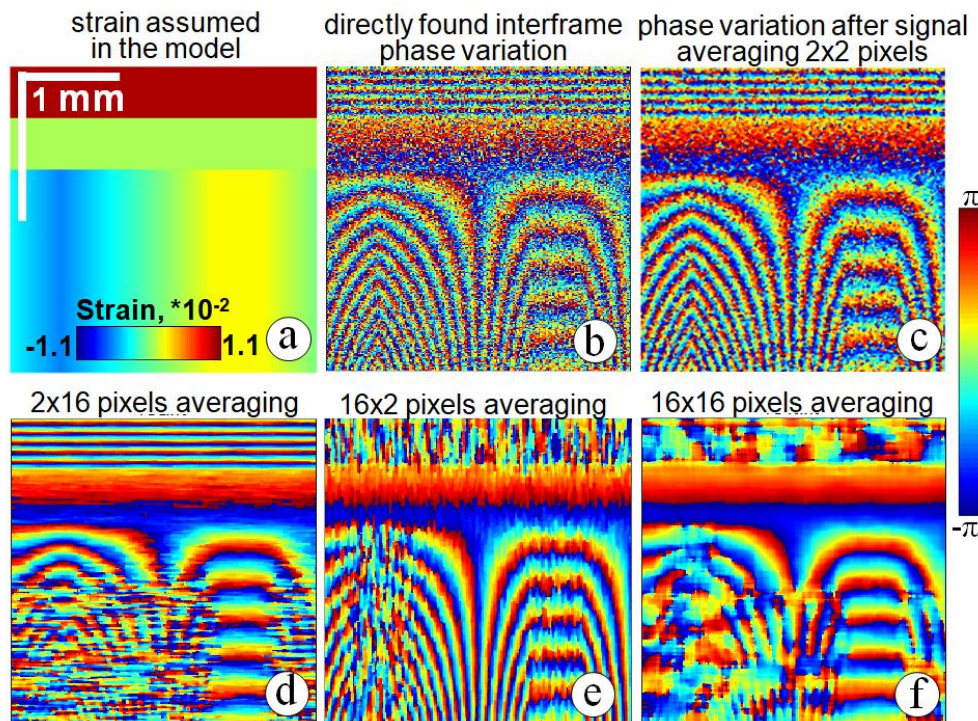


Fig. 1 Demonstration of influence of averaging window size on the resultant phase-variation maps using a numerically simulated example. (a) Interframe strain distribution adopted in the simulations, (b) numerically simulated interframe phase difference without preliminary averaging. Other panels show phase differences after preliminary averaging using various window sizes $W_z \times W_x$ in pixels: (c) 2×2 , (d) 2×16 , (e) 16×2 , and (f) 16×16 . Bigger windows, while being the best in one area, strongly distort the phase difference in others.

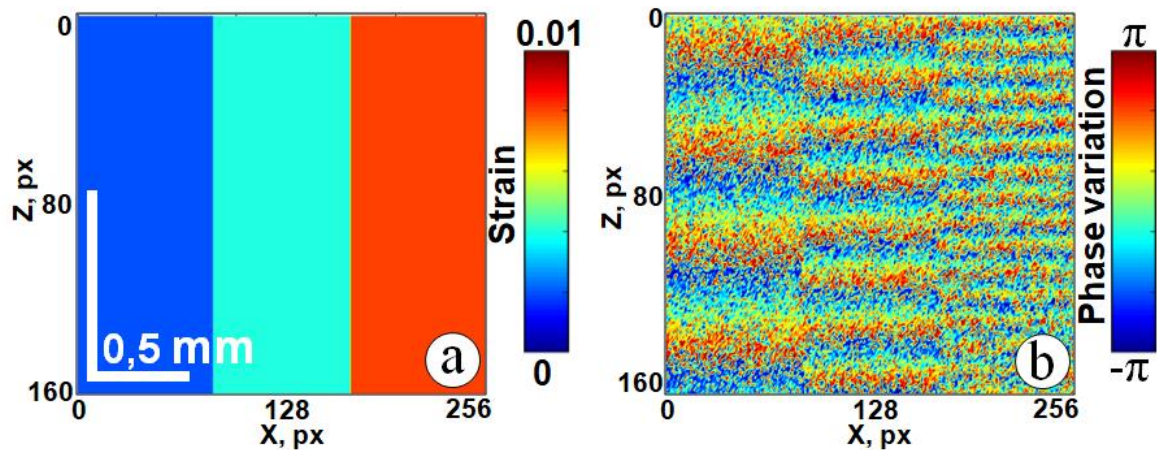


Fig. 2 (a) Adopted for numerical simulations spatially inhomogeneous strain distribution characterized by three regions of axially homogeneous strains of $2 \cdot 10^{-3}$, $4 \cdot 10^{-3}$, and $8 \cdot 10^{-3}$, respectively. (b) The corresponding interframe phase-variation map found without any averaging. The noise level in the simulated scans is SNR = 1.5 dB.

In real conditions the spatial variability of reconstructed strains is *a priori* unknown, so that in different directions and different scan parts the allowable sizes of the averaging window can strongly differ. Usually, to minimize the distorting influence of averaging, but still obtain some noise reduction, one has to use the minimal size of the averaging window 2×2 pixels, although in such a case the noise reduction may be insufficient, although in some scan regions significantly stronger noise reduction can be obtained.

To illustrate the importance of proper choice of the window size for preliminary averaging of complex-valued quantity $a_2(m, j)a_1^*(m, j)$, in Fig. 1 we present a numerically simulated example assuming that the interframe phase variations are produced by the strain field shown in Fig. 1a, where in various scan regions the strain is intentionally chosen either laterally or axially homogeneous. For the simulations shown in Fig. 1, OCT beam is assumed enabling depth-independent lateral resolution, details of the simulation approach are described in Refs. [25, 26]. Sufficiently strong, but quite realistic SNR=3.5 dB is adopted in simulated Fig. 1, the measurement noise being modeled by adding random complex-valued quantities to the complex-valued amplitude of every pixel, so that the directly found via Eq. (1) interframe phase variations are rather noisy as shown in Fig. 1(b). The other panels show the phase-variation maps obtained after averaging the quantity $a_2(m, j)a_1^*(m, j)$ over windows with various sizes 2×2 , 2×16 , 16×2 , and 16×16 pixels. Fig. 2(c) shows that the smallest averaging window 2×2 pixels does not yet distort the shapes of phase-variation isolines, but yields only moderate noise reduction. The other larger windows give much stronger noise reduction in some areas without distortion of isoline shapes, but in other parts of the scan the inappropriately chosen window sizes and shapes produce very strong distortions of the interframe phase variation. In these image parts, the phase-variation

gradients and, consequently, the reconstructed strains obviously will be strongly distorted.

These examples shown in Fig. 1 already qualitatively indicate that, similarly to the adaptive choice of gradient-estimation scale [23], the adaptive choice of the window size for preliminary vector-averaging is also important. To show the importance of the preliminary vector averaging for the strain-estimation quality in more direct quantitative way we consider another example with simpler strain distribution allowing for easier interpretation. Fig. 2(a) shows the assumed strain distribution in the form of three vertical stripes and Fig. 2(b) shows the corresponding interframe phase variation $\Phi(m, j)$ defined by Eq. (2). The size of the scans is 160 pixels axially and 256 pixels laterally, with the axial and lateral distances between the pixel centers, $h_z = 6 \mu\text{m}$ and $h_x = 10 \mu\text{m}$, respectively. As for Fig. 1, the illuminating beam has depth-independent radius $w_0 = 10 \mu\text{m}$ with the central wavelength $\lambda_0 = 1 \mu\text{m}$. The number of scatterers in the scan is 256000, which corresponds to typical density of cells in biotissues. The noise level in the simulated scans is rather high with SNR=1.5 dB.

Next, the three columns in Fig. 3 corresponding to the phase-variation map shown in Fig. 2(b) demonstrate the reconstructed axial strains that are proportional to the axial phase-variation gradients estimated by the vector method [14] for various values of the axial inter-pixel distance $g = 1, 2$, and 4 pixels (in other words, various gradient-estimation scale the role of which is discussed in detail in Ref. [23]). The upper row in Fig. 3 shows the influence of the chosen gradient-estimation scale g without any additional utilization of preliminary vector averaging of quantity $a_2(m, j)a_1^*(m, j)$ directly from the phase variations shown in Fig. 2(a). The bottom row in Fig. 2 corresponds to the same gradient-estimation scales $g = 1, 2$, and 4 pixels, but with additional preliminary vector averaging of quantity $a_2(m, j)a_1^*(m, j)$ over the window 4×4 pixels.

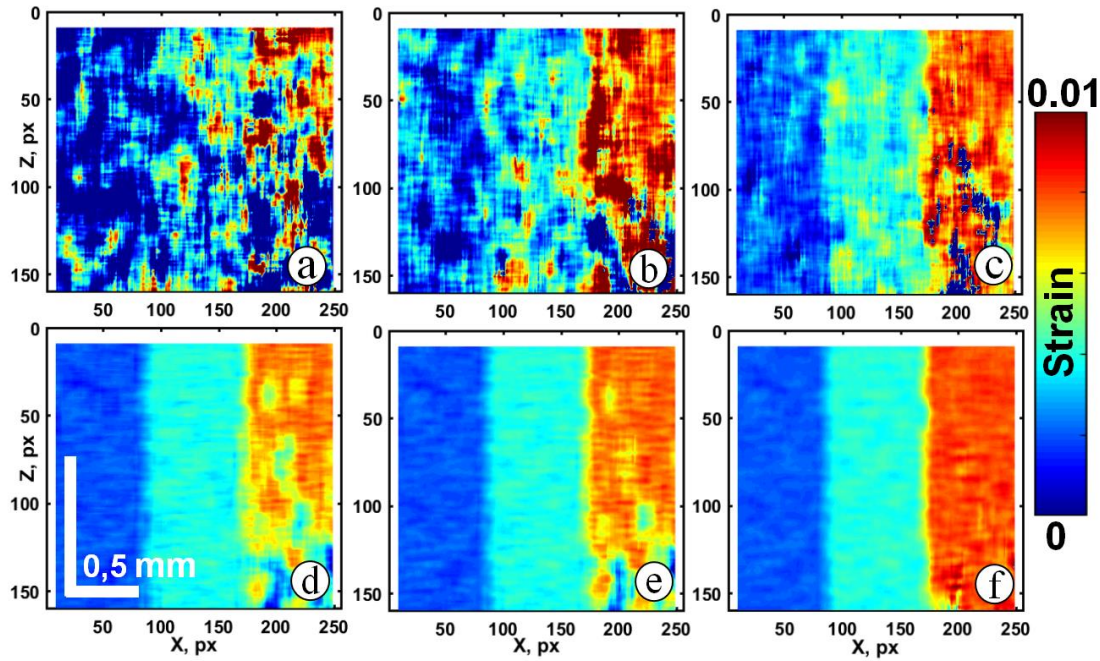


Fig. 3 Reconstructed strain maps based on phase-variation map shown in Fig. 2b for various values of gradient-estimation scale g and averaging-window sizes $W_x = W_z$ of 1×1 (a–c) and 4×4 pixels (d–f). The first column is for $g = 1$, second for $g = 2$, and third column for $g = 4$.

Fig. 3 demonstrates that the quality of the reconstructed strain essentially depends on the processing parameters. It is also clear from this example that the choice of the gradient-estimation scale g earlier discussed in Ref. [23] and the window size $W_x \times W_z$ for preliminary averaging of quantity $a_2(m, j)a_1^*(m, j)$ before estimating the phase-variation gradient may give significant contributions to the improvement of the strain-reconstruction quality.

In the following sections we specifically consider how the window size for preliminary vector averaging affects the quality of strains reconstructed using the vector method [14]. This method is chosen because of such advantages as intrinsic high robustness with respect to various noises and high computational efficiency. The results will be presented in quantitative form based on both numerical simulations like for Fig. 2 and supported by analytical argumentation. Then the method of adaptive choice of the window size $W_x \times W_z$ for preliminary averaging of quantity $a_2(m, j)a_1^*(m, j)$ will be formulated, to complement the earlier obtained results [23] for adaptive choice of gradient-estimation scale g . To make the discussion of these problems clearer, firstly, the main steps of the vector method [23] will be recalled in the next section.

3 Brief Summary of the Vector Method of Strain Estimation

In the vector method of phase-gradient estimation pixelated OCT signals characterized by complex-valued amplitudes $a(m, j) = A(m, j)\exp[i \cdot \phi(m, j)]$ till the last

stage are processed without explicit phase extraction, according to the main steps schematically shown in Fig. 4:

a) The first step is pixel-by-pixel multiplication of the deformed B-scans and complex conjugated reference one:

$$a_2(m, j)a_1^*(m, j) \equiv b(m, j) = B(m, j)\exp[i \cdot \Phi(m, j)], \quad (3)$$

where $\Phi(m, j) = \phi_2(m, j) - \phi_1(m, j)$ and $B(m, j) = A_2(m, j)A_1(m, j)$. The matrix $b(m, j)$ contains interframe phase variations $\Phi(m, j)$.

b) Second, to improve the signal-to-noise ratio (SNR), vector averaging of quantity $b(m, j)$ is performed using a small sliding window $W_z \times W_x$ in size:

$$\bar{b}(m, j) \equiv \hat{B}(m, j)\exp[i \cdot \hat{\Phi}(m, j)] = \sum_{m'=1}^{W_z} \sum_{j'=1}^{W_x} b(m'+m, j'+j), \quad (4)$$

The role of just this averaging step will be considered in detail further.

c) Then for finding the axial phase-variation gradients, the next step is finding the following matrix:

$$d(m, j) = D(m, j)\exp[i \cdot \Psi(m, j)] = \bar{b}(m+g, j)\bar{b}^*(m, j). \quad (5)$$

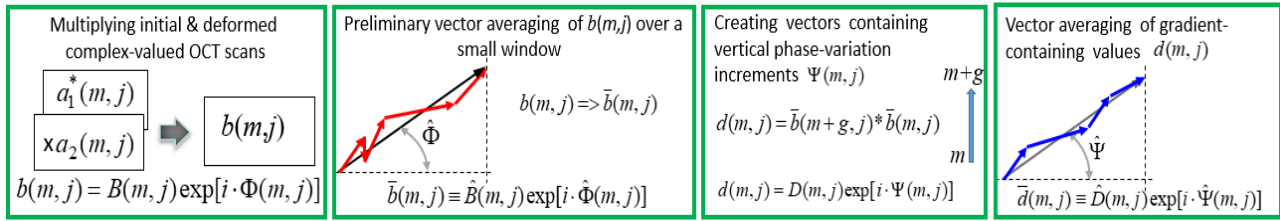


Fig. 4 The main steps of the vector method of strain estimation. All intermediate calculations are performed with complex-valued quantities, and the sought phase-variation gradient is extracted at the final stage.

It contains the phase difference $\Psi(m, j)$ between pixels with axial indices m and $m + g$, where the inter-pixel distance g is the scale, over which the phase-variation gradient is estimated. In the simplest case $g = 1$, however the use of a larger $g > 1$ (if there is no ambiguity in the interframe phase variations on this scale) can significantly improve the quality of strain estimation as was discussed in Ref. [23].

d) To further suppress noise in the reconstructed field of axial gradients of interframe phase variations, vector averaging of quantity $d(m, j)$ can be used with a window of size $M \times J$ pixels:

$$\begin{aligned} \bar{d}(m, j) &\equiv \hat{D}(m, j) \exp[i \cdot \hat{\Psi}(m, j)] = \\ &= \sum_{m'=1}^M \sum_{j'=1}^J d(m'+m, j'+j). \end{aligned} \quad (6)$$

The size $M \times J$ of this window usually is significantly larger than in stage (b), because the scale, over which gradient $\Psi(m, j)$ noticeably changes, is usually much larger than the scale over which the interframe phase variation $\Phi(m, j)$ noticeably changes.

e) Finally, the sought strain was expressed via the phase-variation gradient $\hat{\Psi}(m, j)$ as:

$$\hat{s}_z = \hat{\Psi}(m, j) / (2k_0 h_z g), \quad (7)$$

where $k_0 = 2\pi / \lambda_0$ is the central wavenumber of the OC-source spectrum.

In what follows step (b) will be called preliminary vector averaging and step (d) – final vector averaging.

4 Influence of Window Size for Preliminary Vector Averaging on the Axial Strain Estimation: Numerical Evaluation and Analytical Arguments

To numerically evaluate how the vector averaging of quantity $b(m, j) \equiv a_2(m, j)a_1^*(m, j)$ affects the quality of the strain estimation, we used a pair of simulated B-scans, for which the interframe strain adopted in the simulation and the corresponding phase difference $\Phi(m, j)$ are shown in Fig. 2. We recall that SNR for the simulated scans was equal to 1.5 dB and other simulation parameters were indicated in the discussion of Fig. 2.

Based on the simulated data, strain is calculated according to Eqs. (3)–(7). The strain is calculated for different averaging window sizes in Eq. (4). The gradient-estimation scale g in Eq. (5) is set to 4 and the final averaging in Eq. (6) for quantity $\bar{d}(m, j)$ (the argument $\hat{\Psi}(m, j)$ of which is proportional to strain) is performed using the final averaging window 16×16 pixels.

Then we calculate the dependence of the found mean strain value S_{mean} and the standard deviation (STD) for strain on the preliminary averaging window size. In the discussed simulated example shown in Fig. 2 there are three regions with three levels of homogeneous strain, for which the isophase lines are laterally strictly horizontal, so that the results of averaging in the axial and lateral directions should essentially differ. To clearly show this difference, while incrementing the window size along one axis the window size along another axis was set to fixed value 2. The numerically obtained dependences are shown (in black lines) in Fig. 5 as a function of W_x and Fig. 6 as a function on W_z .

It is clear from Fig. 5 that for horizontally homogeneous interframe phase variation, the estimates of S_{mean} in Fig. 5 remain close to the value adopted in simulations and the deviation $\text{STD}(W_x)$ monotonically decreases with increasing W_x , which is intuitively expectable. In contrast, the dependences on the vertical scale W_z of preliminary vector averaging are rather non-trivial. For the estimated $S_{\text{mean}}(W_z)$ in Fig. 6 (upper row), a clear drop is visible for $W_z > g$ (we recall that $g = 4$ for this example). The behavior of $\text{STD}(W_z)$ (see Fig. 6, bottom row) is essentially non-monotonic with a minimal deviation for a certain W_z , for which its relation to g will be discussed in the following sections.

To elucidate the origin of these numerically revealed dependences we may present the following analytical argumentation. Let us first consider a case of positive homogeneous strain s_z that is independent of the depth, as well as of the lateral coordinate, so that the interframe phase difference increases linearly with depth as in the discussed examples. However, even in case of such horizontally uniform strain s_z we consider a somewhat more general situation, in which the displacements of scatterers Δz may depend not only on depth z , but may be accompanied by a vertical translation shift dependent on x (such that the iso-phase lines are inclined rather than horizontal as in Fig. 2).

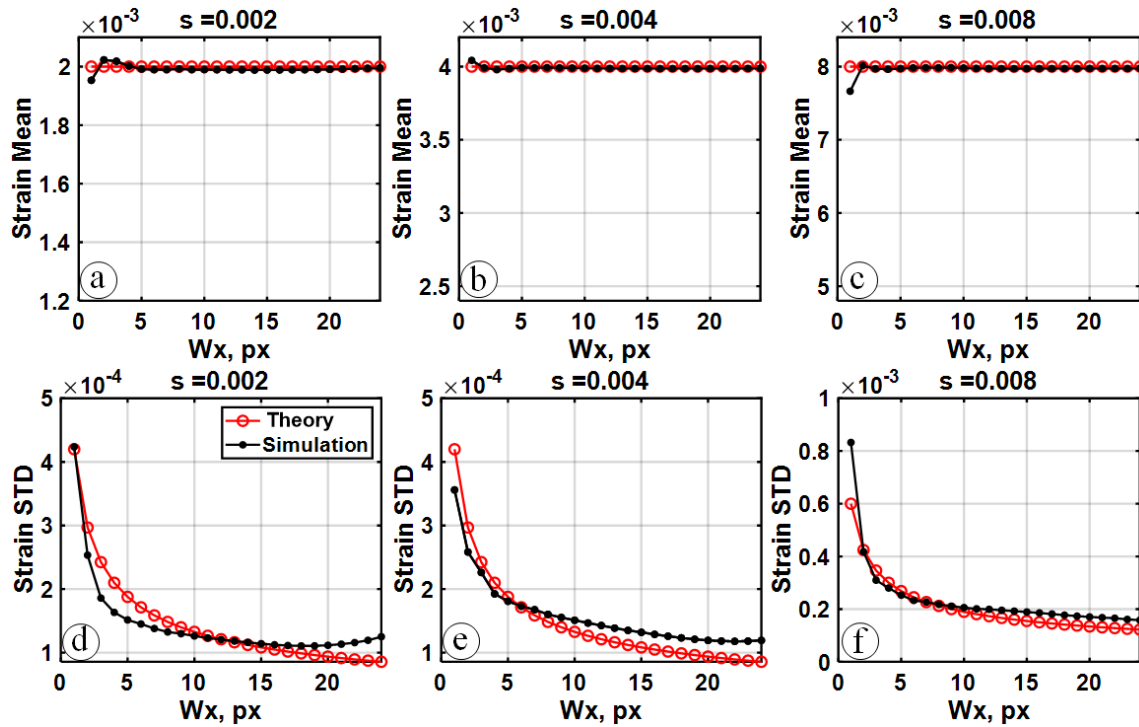


Fig. 5 Numerical evaluation of the influence of preliminary averaging window size W_x on the axial strain estimation for the horizontally homogeneous strains and the corresponding interframe phase variations shown in Fig. 2. The (a), (b), and (c) panels show the estimated strain values found for the fixed gradient-estimation scale $g = 4$, fixed vertical size $W_z = 2$ of the averaging window and variable horizontal size W_x . The (d), (e), and (f) panels show the standard deviation for the estimated strain as a function of W_x .

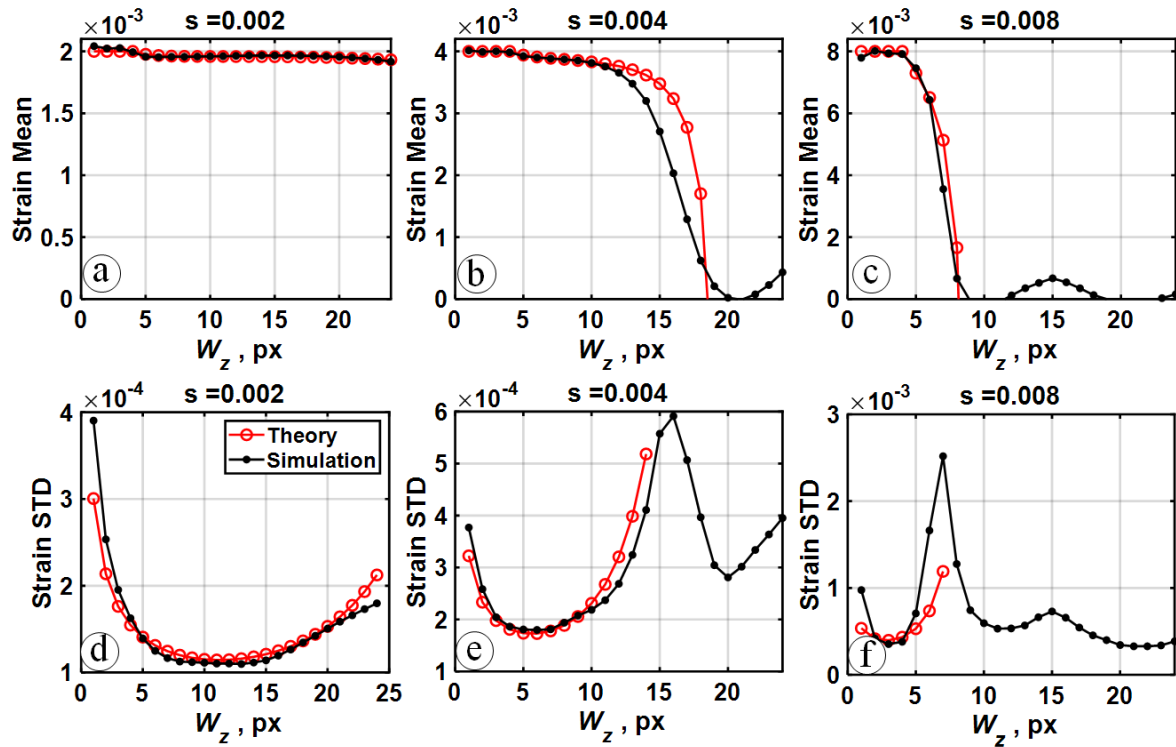


Fig. 6 Numerical evaluation of the influence of preliminary averaging window size W_z on the axial strain estimation for the horizontally homogeneous strains and the corresponding interframe phase variations shown in Fig. 2. The (a), (b), and (c) panels show the estimated strain values found for the fixed gradient-estimation scale $g = 4$, fixed horizontal size $W_x = 2$ of the averaging window and variable vertical size W_z . The (d), (e), and (f) panels show the standard deviation for the estimated strain as a function of W_z .

Thus, for such interframe displacements of the form $\Delta z = s_z z + s_x x$, the interframe phase difference is $\Phi = 2k_0 \Delta z = 2k_0 (s_z z + s_x x)$. For pixilated scans this phase difference can be written as $\Phi(m, j) = 2k_0 [(h_z s_z m + h_x s_x j)]$, where h_z and h_x are the axial and lateral distances between neighboring pixel centers; quantities m and j are the axial and lateral pixel indices.

We also assume that some measurement noise is present and is represented by adding to each pixel a complex-valued random quantity characterized by a Gaussian distribution with zero mean and standard deviation σ . For these assumptions, the registered pixilated OCT signal can be written as $a(m, j) = S(m, j) + n(m, j)$, where $S(m, j)$ is the true OCT signal without the noise and $n(m, j)$ is the additive Gaussian noise characterized by a correlational function $\langle n(m_1, j_1) n^*(m_2, j_2) \rangle = \sigma^2 \delta(m_2 - m_1) \delta(j_2 - j_1)$, where δ is the Kronecker symbol. The complex-valued signal before deformation can be written as $S_1(m, j) = A \cdot \exp[i\varphi(m, j)]$, where the initial phases $\varphi(m, j)$ are determined by random positions of scatterers and the amplitudes are taken equal for simplification. After deformation it takes the form $S_2(m, j) = A \cdot \exp\{i\varphi(m, j) + i\Phi(m, j)\}$. Performing all the steps of strain calculation for the signal part when $a(m, j) = S(m, j)$ using Eqs. (3)–(6) one obtains the following averaged value $\bar{d}$ of quantity defined by Eq. (6):

$$\bar{d} = A^4 \text{sinc}^2(k_0 s_z h_z W_z) \cdot \text{sinc}^2(k_0 s_x h_x W_x) \cdot \exp(i2k_0 s_z h_z g), \quad (8)$$

where the sinc-function in Eq. (8) is $\text{sinc}(y) = \sin(y)/y$. As can be seen from Eq. (8), averaging over a window with sizes W_z and W_x leads to decrease in amplitude of $|\bar{d}|$ with zero value reached for $k_0 s_z h_z W_z = \pi$ or $k_0 s_x h_x W_x = \pi$.

Then the calculation of the mean strain estimate $\hat{S}_z$ and its standard deviation $\text{STD}(\hat{S}_z)$ was performed based on Eqs. (3)–(7) in the presence of noise (i.e., for $a(m, j) = S(m, j) + n(m, j)$) with account for the above formulated noise properties and assuming sufficiently high output (i.e., after Eq. (6)) signal-to-noise ratio for estimating strain $\hat{S}_z$ and its standard deviation $\text{STD}(\hat{S}_z)$.

It appears that the mean value for estimated strain $\hat{S}_z$ is unbiased and equal to the actual strain S_z if $W_z \leq g$. However, when W_z is greater than scale g , over which the phase-variation gradient is calculated, the performed

analysis indicated the appearance of noise-induced bias $\Delta(\hat{S}_z)$ in the estimation of strain $\hat{S}_z$:

$$\Delta(\hat{S}_z) = \begin{cases} 0, & \text{for } W_z \leq g \\ -s_z \frac{\sigma^2}{A^2} \left(2 + \frac{\sigma^2}{A^2} \right) \frac{\text{sinc}(2k_0 s_z h_z g)}{\text{sinc}^2(k_0 s_z h_z W_z) \text{sinc}^2(k_0 s_x h_x W_x)} \frac{W_z - g}{W_z^2}, & \text{for } W_z > g. \end{cases} \quad (9)$$

These approximate analytical estimates are shown by red lines with empty circles in Figs. 5 and 6. The bias of the estimated strain for $W_z > g$ described by Eq. (8) is clearly seen in the upper row of Fig. 6. It is clear that the analytical estimates well agree with the numerical results. The reason of the above-mentioned bias in the estimated $\hat{S}_z$ is due to the fact that for too large averaging with $W_z > g$, the noise acquires some correlation within the gradient-estimation scale g . Therefore, W_z must be chosen smaller than the gradient-estimation parameter g , for which the adaptive choice is discussed in Ref. [23]. In view of the independence of strain of the lateral coordinate for the considered strain shown in Fig. 2, there is no similar limitation on the lateral size W_x of the window for preliminary averaging (see the lower row in Fig. 5).

For the same conditions and assumptions, calculations based on Eqs. (3)–(7) yield the following functional dependence for the standard deviation $\text{STD}(\hat{S}_z)$ of strain in the presences of noise $n(m, j)$:

$$\text{STD}(\hat{S}_z) \propto \frac{\sigma^2}{A^2} \left(2 + \frac{\sigma^2}{A^2} \right) \cdot \frac{1}{k_0 h_z g \sqrt{M J} \cdot \text{sinc}^2(k_0 s_z h_z W_z) \cdot \text{sinc}^2(k_0 s_x h_x W_x) \cdot \sqrt{W_z W_x}}. \quad (10)$$

In this analytical estimate we focus on the functional dependences rather than exact estimation of pre-factor in this equation. For clearer comparison with numerical results this pre-factor can easily be adjusted. The corresponding superposition of numerical (black color) and analytically found (red color) curves for $\text{STD}(\hat{S}_z)$ is shown by red lines in the bottom rows in Figs. 5 and 6.

Again for lateral preliminary averaging the curves of the two types (bottom row in Fig. 5) show monotonic decrease in $\text{STD}(\hat{S}_z)$ approximately as $1/\sqrt{W_x}$ as follows from Eq.(10) for $s_x = 0$ corresponding to Fig. 2 with strictly horizontal isophase lines. However, for inclined isophase lines (i.e. for $s_x \neq 0$), function $\text{sinc}^2(k_0 s_x h_x W_x)$ in Eq. (10) indicates that, due to horizontally varying phase, the result of lateral vector averaging should become non-monotonic as a function of W_x .

For the vertical averaging, the dependence of $\text{STD}(\hat{S}_z)$ on the scale W_z of vertical preliminary-averaging is essentially non-monotonic (bottom row in Fig. 6) because of the vertically inhomogeneous phase

variation the axial gradient of which is determined by the axial strain level s_z .

Eq. (10) makes it possible to predict the sizes W_z and W_x of the preliminary-averaging window, for which the standard deviation $\text{STD}(\hat{s}_z)$ is minimal. It follows from Eq. (10) that minimal $\text{STD}(\hat{s}_z)$ is attained in the maximum of function $f(W_z, W_x) = \text{sinc}^2(k_0 s_z h_z W_z) \cdot \text{sinc}^2(k_0 s_x h_x W_x) \cdot \sqrt{W_z W_x}$ that enters the denominator in Eq. (9). The minima of $\text{STD}(\hat{s}_z)$ as function of W_z and W_x are attained independently of each other. Using the notation $\alpha = 2k_0 s_z h_z W_z$ it is possible to find the maximum of function $f(\alpha) = \text{sinc}^2(\alpha/2) \cdot \sqrt{\alpha}$. The shape of this function shown in Fig. 7 indicates that its maximum occurs at $\alpha \approx \pi/2$, therefore, $\text{STD}(\hat{s}_z)$ has minimum near this point. In what follows criterion $\alpha \approx \pi/2$ will be used for determining the optimal window sizes for preliminary averaging in both directions.

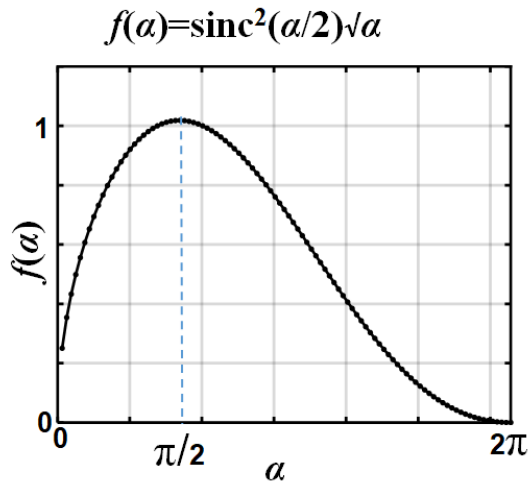


Fig. 7 The shape of function $f(\alpha) = \text{sinc}^2(\alpha/2) \cdot \sqrt{\alpha}$ that determines the averaging-window sizes W_z and W_x , for which $\text{STD}(\hat{s}_z)$ reaches minimum. The latter corresponds to $f(\alpha)$ maximum that is reached around $\pi/2$.

Further, when choosing the optimal W_z one also should take into account the absence of bias for the estimated $\hat{s}_z$, namely, according to Eq. (9) it should be $W_z < g$, where parameter g is the scale used in Eq. (5) to estimate the axial phase-variation gradient. Optimization of parameter g was considered in detail in paper [23]. Although the use of larger values $g > 1$ is favorable for enhancing the gradient-estimation accuracy, in the presence of phase wrapping (when numerous rainbow stripes are present in the strain-variation map within the visualized region as in Fig. 2(a) along the vertical direction), the axial phase difference for too big g experiences jumps of 2π rad. (phase wrapping) and, consequently, the estimated axial strain changes the sign. Using this criterion of the incorrect sign change for the

estimated strain, the condition of optimal g_{opt} can be formulated as $g_{opt} < \lambda_0 / (4s_z h_z)$. Then, recalling that $\alpha = 2k_0 s_z h_z W_z$ and the optimal $\alpha \approx \pi/2$, we obtain that for W_z , the optimal value can be chosen as $W_z^{opt} = g_{opt} / 2 = \lambda_0 / (8s_z h_z)$.

If the isophase lines are inclined like in Fig. 1 in the presence of strain component $s_x \neq 0$, then the same condition $\alpha \approx \pi/2$, similarly to W_z , can be used in the lateral direction and the optimal value for the horizontal window size can be obtained, $W_x^{opt} < \lambda_0 / (8s_x h_x)$. If $s_x = 0$ and the interframe phase variation does not depend on the lateral coordinate (like shown in Fig. 2(a)), there is no such limitation on the optimal W_x , so that the only limiting condition on its choice is the desirable spatial resolution in the reconstructed strain maps.

5 Algorithm for Automatic Adaptive Choice of Preliminary Averaging Window Size

The above presented analysis allows one to formulate a practical algorithm for the adaptive selection of window sizes $W_{x,z}$ for preliminary vector averaging given by Eq. (4).

The first step is calculation of vertical and horizontal phase gradients with Eqs. (3)–(6). Note that for lateral gradient $\hat{\Psi}$ in Eq. (6) it must be calculated with a horizontal step $g^{(x)}$ instead of vertical step $g^{(z)}$. In terms of auxiliary parameter α considered in Fig. 7, the criterion of the optimal window size for preliminary averaging was formulated in the previous section as $\alpha = \pi/2$. It was demonstrated in Section 4 that in the axial direction z , this criterion corresponds to condition $W_z^{opt} = g_{opt}^{(z)} / 2$, where $g_{opt}^{(z)}$ is the optimal gradient-estimation scale g_{opt} in the axial direction. It was discussed in detail in Ref. [23] that the optimal value of g_{opt} for estimating phase-variation gradient corresponds to such a scale g in Eq. (5), over which the phase difference $\Psi(m, j)|_{g=g_{opt}}$ tends to wrapping (i.e., experiences a jump by 2π rad). Notice, that in Eq. (6) phase difference $\Psi(m, j)|_g$ is considered for the scale g in the axial direction, but very similar arguments also relate to the lateral direction in case of laterally inhomogeneous interframe phase variations. Thus, for the lateral direction x , similarly to the axial one, the optimal window size should be found as $W_x^{opt} = g_{opt}^{(x)} / 2$.

After finding the so-defined optimal scales $g_{opt}^{(z)}$ and $g_{opt}^{(x)}$ for estimating phase-variation gradients in every image pixel (m, j) , we obtain the mask of optimal sizes of preliminary-averaging windows in the axial and lateral directions, $W_{z,x}(m, j) = g_{opt}^{(z,x)} / 2$. If the so-found window

size is not integer, a closest smaller integer value must be taken.

The next step is the realization of preliminary vector averaging of quantity $b(m, j)$ via Eq. (3) to find the averaged matrix $\bar{b}(m, j)$ using the determined sizes $W_{x,z}^{opt}(m, j)$ as described above. Next, quantity $d(m, j)$ containing the interframe phase-variation gradient $\Psi(m, j)$ can be estimated using Eq. (5) and the final averaging of $d(m, j)$ is performed using Eq.(6) to obtain $\bar{d}(m, j)$ and $\hat{\Psi}(m, j)$.

Also using the so-found less noisy data $\bar{b}(m, j)$ one may iteratively repeat the above-mentioned procedures for estimating the scales $g_{opt}^{(x,z)}$ [23] and, therefore, find refined sizes of the averaging window $W_{x,z}^{opt} = g_{opt}^{(x,z)} / 2$, which should yield improved estimates of the sought strain.

Examples of strain estimation with utilization of the above-described adaptive preliminary averaging are given in the following sections for both simulated and real OCT data.

6 Adaptive Preliminary Averaging: Simulated Examples

In the following examples we again used the approach described in details in Refs. [25, 26]. for simulating images for spectral OCT systems with “typical” parameters – a weakly focused illuminating beam (having a Gaussian profile with radius = 16 μm weakly changing with depth), the central wavelength of 1.3 μm (in the air) and a spectrum width of 90 μm . Each A-scan consisting of 256 pixels covering 2 mm in depth (in air) is formed by superposition of 256 complex-values spectral components. The lateral spacing of A-scans is 16 μm , and horizontally the B-scans consist of 200 A-scans. Discrete point-like scatterers were randomly distributed in the visualization area with an average distance between them of $\sim 5 \mu\text{m}$, which corresponds to the typical cell density in biological tissues. The measurement noise was modeled by adding to each pixel random complex-valued number with Gaussian statistics. The noise level in the so-simulated scans corresponded to a signal-noise ratio (SNR) of 3.5 dB. For such fairly strong noise, the optimization of estimation of phase gradient and strain is particularly important.

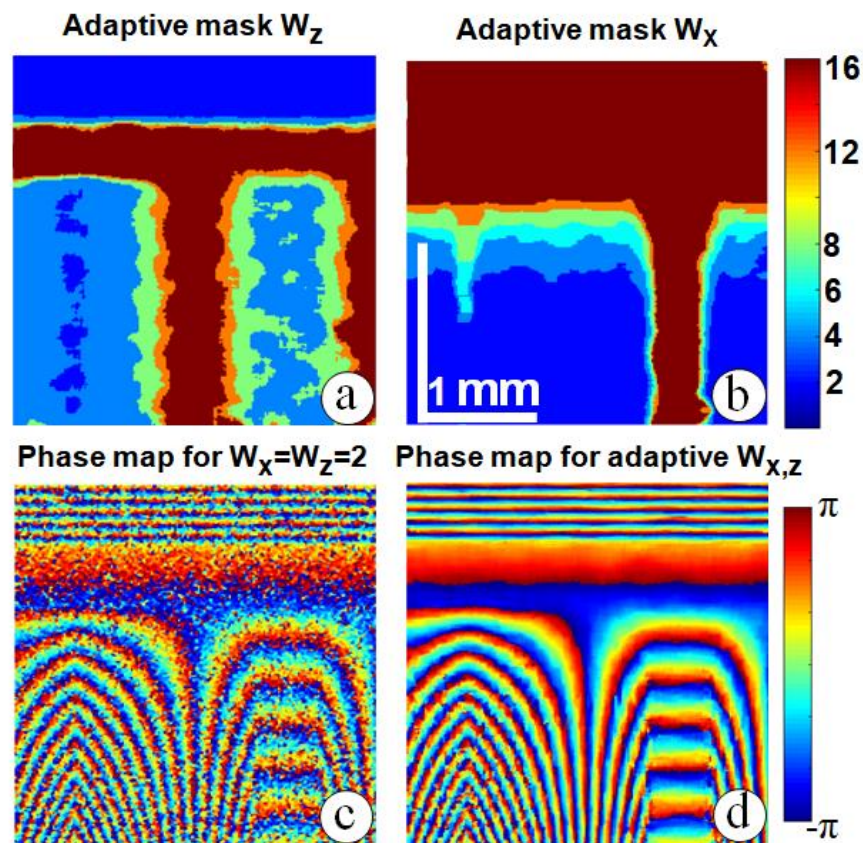


Fig. 8 Color coded adaptively chosen window sizes $W_{x,z}$ in the range 2...16 for preliminary averaging and illustration of the phase-variation map improvement. (a) Adaptively chosen W_z , (b) adaptively chosen W_x , (c) interframe phase difference for preliminary averaging with a fixed window 2×2 pixels, (d) interframe phase difference for preliminary averaging with adaptively chosen W_z and W_x shown in panels (a) and (b). The effect of noise reduction for adaptive averaging is clearly visible.

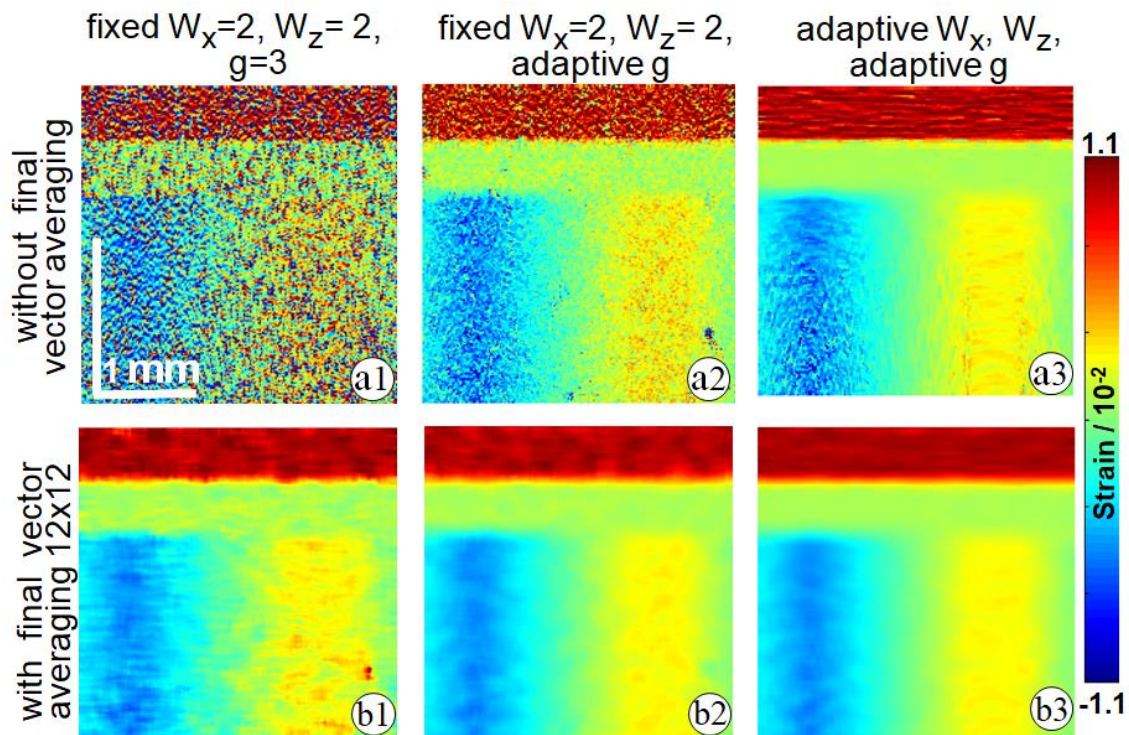


Fig. 9 Comparison of strains calculated for various combinations of averaging parameters and gradient-estimation scale g . The (a1) and (b1) panels are for fixed preliminary averaging window size $W_z = W_x = 2$ and fixed $g = 3$. The (a2) and (b2) panels are for fixed window $W_z = W_x = 2$ and adaptively chosen g . The (a3) and (b3) panels are for adaptive choice of all three parameters W_z , W_x , and g . (a1), (a2), and (a3) panels – unaveraged strains; (b1), (b2), and (b3) panels – averaged strains.

The simulated distribution of strains, according to which the interframe displacement of scatterers was performed, and the corresponding interframe phase-variation map are shown in Fig. 1. Such a strain distribution was chosen with an idea to present a large number of possible scenarios in one image to demonstrate the capabilities of the method. Strong horizontally homogeneous strains typical for a soft silicone calibration layer which is used for mapping the elastic properties of biological tissues [27, 28] were modeled in the top layer. The modeled weak homogeneous strains in the middle layer of Fig. 1a and surrounding intense rather complex horizontally non-uniform strains of positive and negative signs are also typical of some real experiments, e.g., related to visualization of thermomechanically induced strains in samples of eye cornea and cartilages [29] were also modeled.

There are several different characteristic areas in the phase difference in Fig. 1(b): dense in vertical direction and homogeneous in horizontal (top layer), slowly varying in vertical direction and homogeneous in horizontal (middle layer), almost vertical lines in the bottom middle part and inclined isophase lines in many other areas.

To the so-simulated data, the algorithm with adaptive preliminary averaging window size described in the previous section was applied. The resulting masks for chosen axial and lateral window sizes W_x and W_z are presented in Figs. 8(a) and 8(b), respectively. These

masks are not strictly regular. The irregularities are due to the fact that they are chosen for the noisy simulated data. Comparison of the use of the proposed optimized averaging method and averaging with a fixed window size of 2×2 pixels is shown in Figs. 8(c) and Fig. 8(d). The adaptive averaging shows significant reduction of noise.

Fig. 9 demonstrates comparison of the reconstructed strain maps obtained for several combinations of the gradient-estimation scale g and parameters $W_{x,z}$ for preliminary averaging. The upper row in Fig. 9 demonstrates the strain distributions found using only preliminary vector averaging of the interframe phase variation (given by Eq. (5)), whereas the lower row shows the strain maps after final vector averaging of quantity $d(m, j)$ containing phase-variation gradients (using Eq. (6)). The latter procedure is made for the same averaging-area size 12×12 pixels in all the cases. The left column in Fig. 9 is obtained with preliminary averaging using small fixed window $W_z = W_x = 2$ pixels and fixed $g = 3$; the middle column is for the fixed window $W_z = W_x = 2$ and adaptively chosen g ; and in the right column all three quantities W_z , W_x , and g are chosen adaptively.

It is important to emphasize that, since the window sizes W_z and W_x for adaptive preliminary averaging are closely linked to the adaptive choice of gradient-estimation scale g , in Fig. 9 we compare all 3 cases: (i) when neither $W_z \times W_x$, nor g are optimized, (ii) when only g is optimized, and (iii) when both parameter g and

the size $W_z \times W_x$ of the preliminary averaging are optimized. The strain distribution obtained with all parameters chosen adaptively demonstrates appreciably better correspondence with the simulated strain distribution shown in Fig. 1(a). The main improvement is seen in the upper layer, where the strain is maximal the optimized gradient-estimation scale g cannot be made larger than 3 pixels and, therefore, improving in SNR can only be achieved by means of preliminary averaging over a larger window.

7 Adaptive Preliminary Averaging: Real Data Examples

This section describes the application of preliminary averaging with adaptively chosen parameters to process OCT-scans obtained in contact mode for mechanically-produced deformations of a three-layer structure consisting of two silicone layers and a cartilage sample placed between them similarly to studies described in Ref. [30]. The OCT system enabled B-scans of 2 mm in depth (in the air) and 4 mm in lateral direction. Examples of registered structural image and the interframe phase difference are presented in Fig. 10. Rapid axial variation of the interframe phase difference in the upper soft silicone layer in Fig. 10 is similar to the upper layer in the simulated phase difference in Fig. 1b. Cartilage is much stiffer than silicone, therefore, in cartilage the interframe phase difference varies slower with depth and can be averaged over larger areas.

In the following Fig. 11, results similar to the model Fig. 8 are presented. Figs. 11(a) and 11(b) are the adaptively chosen masks for axial and lateral averaging sizes W_z and W_x . Notice that the boundaries separating regions with different values W_z and W_x may look somewhat more irregular than the variations in the density of isolines in the phase map shown in Fig. 10(b). These irregularities are due to noises, the influence of which causes fluctuations in the estimated parameters $g_{opt}^{(z)}$ and $g_{opt}^{(x)}$ (i.e., the estimated distances over which

the interframe phase-variation experiences wrapping in z - and x -directions). Correspondingly, the estimated averaging sizes $W_{x,z} = g_{opt}^{(x,z)} / 2$ also fluctuate.

Figs. 11(c) and 11(d) show the phase difference calculated with fixed averaging 2×2 and adaptive averaging. It is worth noting that although in the bottom part the masks (Figs. 11(a) and 11(b)) have irregular structure and might have lower values than optimal due to high level of noise, this fact does not spoil strain estimation compared to fixed 2×2 window but rather does not improve the results, which is not critical.

The comparison of strain maps similar to Fig. 9 for nonadaptive vs. adaptive averaging procedures is shown in Fig. 12. Improvement in the reduction of noise level is quite noticeable especially in comparison with strain shown in Fig. 12(a1) and found for fixed gradient-estimation scale $g=3$ and 2×2 window for preliminary averaging without final averaging via Eq. (6). While strain is completely masked by the noise in Fig. 12(a1), it becomes better visible for adaptive parameter g in Fig. 12(a2) and in Fig. 12(a3) where adaptive g and adaptive preliminary averaging are combined yet without final averaging via Eq. (6). The additional use of final averaging in the lower row of Fig. 12 shows noticeable quality improvement in comparison with the upper row. Thus, Fig. 12 clearly demonstrates that the adaptive choice of both the gradient-estimation scale g and the preliminary averaging window size is very important for improving the strain-mapping quality in OCT-elastography.

8 Discussion and Conclusions

This study for the first time considers an important question for strain reconstruction in phase-sensitive OCT elastography – adaptive preliminary vector averaging of interframe phase difference. Averaging of phase difference is usually performed over a small fixed window to resolve the smallest scale of interframe phase variations in phase-sensitive OCT scans.

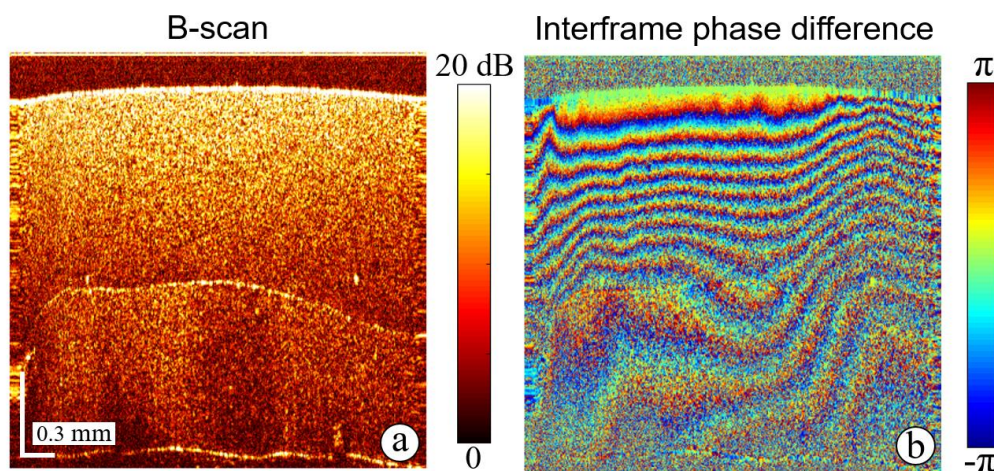


Fig. 10 OCT visualization of a sandwich structure consisting of a cartilage sample between two silicone layers. (a) Structural B-scan, (b) interframe phase difference corresponding to significantly larger deformations in the reference silicone than in cartilage.

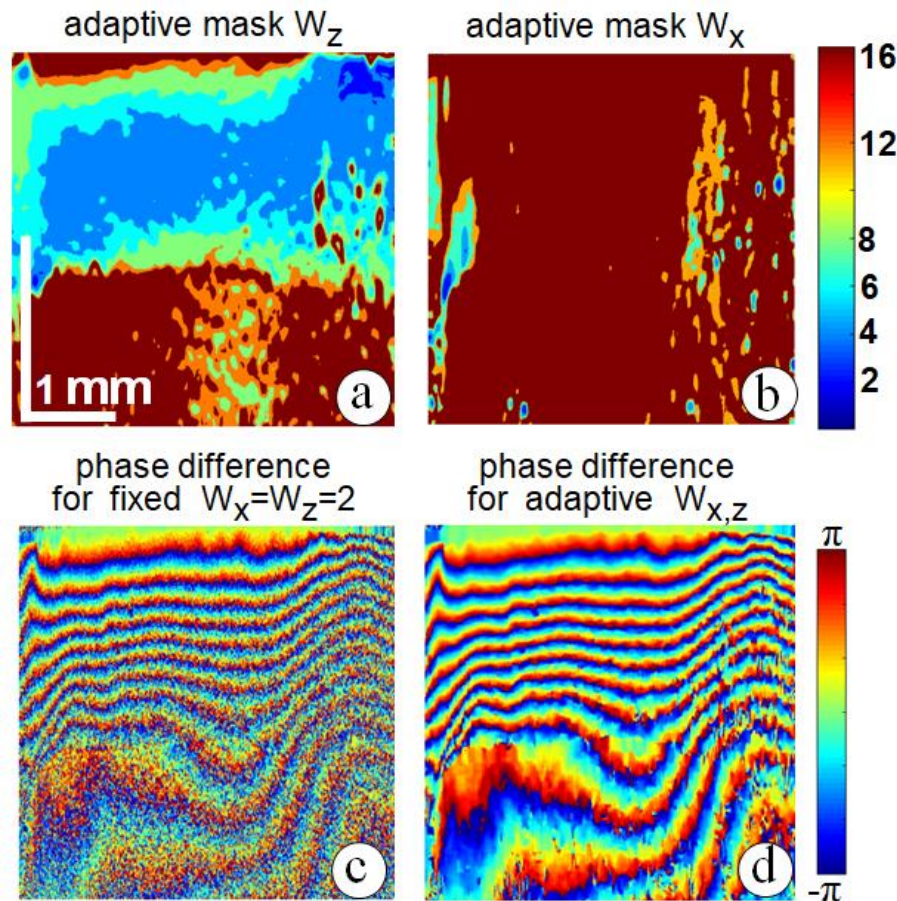


Fig. 11 Panels (a) and (b) show adaptively chosen axial and lateral averaging-window sizes. Panels (c) and (d) show interframe phase difference calculated with fixed averaging window 2×2 pixels (c) and adaptive averaging (d).

The use of a bigger preliminary-averaging window can greatly improve the strain estimation quality. However, such larger averaging windows can be used without reduction in the useful signal only in image regions with sufficiently smooth spatial variation of interframe phase difference. Therefore, adaptive automatic choice of the preliminary-window size is proposed. It is demonstrated the properly performed preliminary averaging may additionally noticeably enhance the quality of strain mapping.

The recent study [23] considered another important issue for mapping strains in phase-sensitive OCT – the adaptive choice of scale g , over which the interframe phase-variation gradients are estimated. In the present study we show that the adaptively chosen window sizes W_z and W_x for preliminary averaging are closely related to the optimal gradient-estimation scales, $W_{x,z} = g_{opt}^{(x,z)} / 2$. The maximal allowable size of parameter $g_{x,z}$ is limited by the lateral and axial variability of interframe phase variations, namely by the scales, over which wrapping of interframe variations occurs.

The results of Ref. [23] and the present study indicate that the quality of strain mapping initially increases $\propto g$ and $\propto \sqrt{W_{x,z}}$. Notice that quite often the interframe

phase variations are significantly weaker dependent on the lateral coordinate than in the axial direction. Consequently, the averaging size W_x may be increased in comparison with W_z to additionally enhance strain mapping quality by preliminary averaging.

Adaptive selection of scales W_z and W_x for preliminary-averaging is performed for each direction separately using basically similar procedures to the choice of optimal gradient estimation scale in Ref. [23]. In both directions, the selection of $W_{x,z}$ is based on estimating the scale, over which interframe phase variations experience wrapping (i.e., a jump by π rad.). We recall that this scale determines the optimal values of scales $g_{x,z}^{opt}$ for estimating gradients of interframe phase variations [23]. In the previous sections we presented numerical and analytical argumentation that the optimal averaging-window size $W_{x,z}^{opt}$ should be chosen so that change in the interframe phase variation is around $\pi/2$ in the lateral or axial directions, respectively, which means that $W_{x,z}^{opt} = g_{x,z}^{opt} / 2$.

Here, it is important to emphasize a non-trivial result of the present study concerning the possibility to use only optimization of $W_{x,z}$ with some fixed $g_{x,z}$ or vice versa.

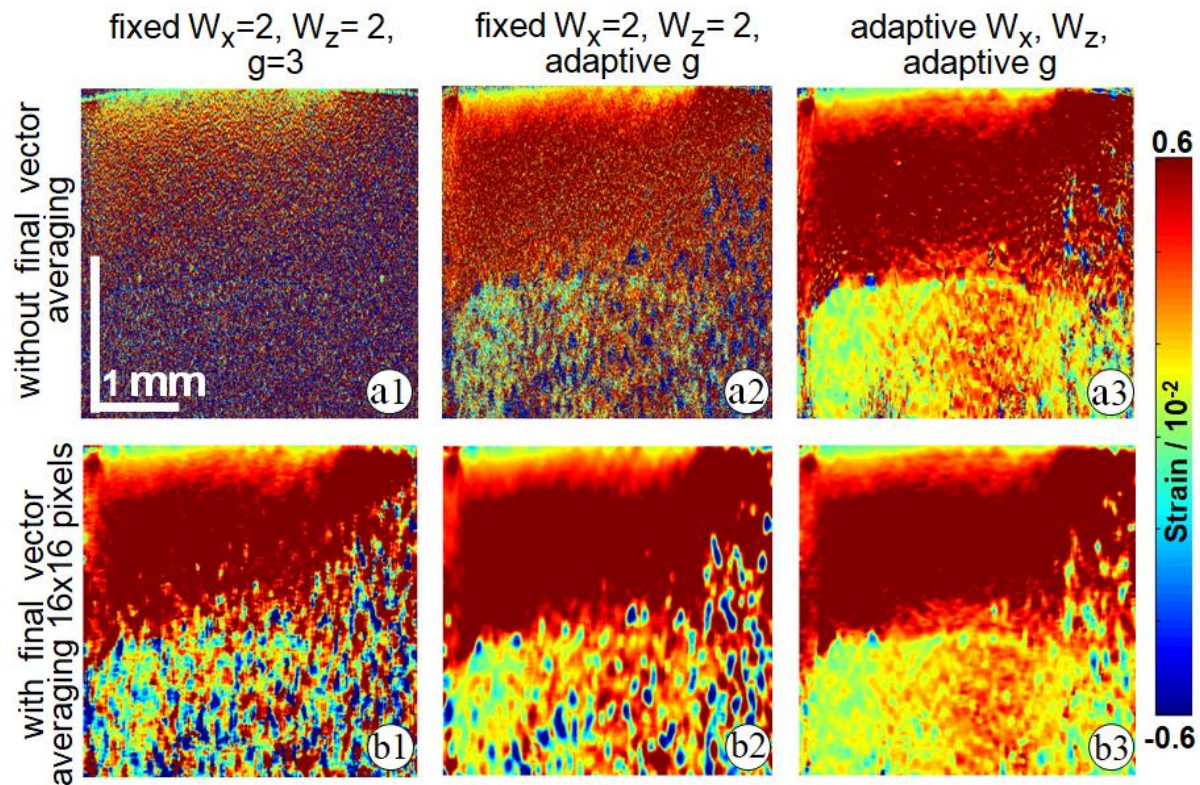


Fig. 12 Comparison of strains calculated using various averaging procedures. The (a1) and (b1) panels are for fixed preliminary averaging window size $W_z=W_x=2$ and fixed $g=3$. The (a2) and (b2) panels are for fixed window $W_z=W_x=2$ and adaptively chosen gradient-estimation scale g . The (a3) and (b3) panels are for the adaptive choice of all three parameters W_z , W_x , and g . The (a1), (a2), and (a3) panels are for the absence of final averaging via Eq. (6) and panels (b1), (b2), and (b3) panels with final averaging.

Namely, the gradient estimation scale $g_{x,z}$ may be chosen adaptively and used for some fixed preliminary-averaging window with a reasonably small size $W_{x,z}$ (say, 2×2 pixels) as was considered in Ref. [23]. Certainly, in this case the noise reduction would not be as efficient as possible, but optimization of only gradient-estimation scales $g_{x,z}$ would not cause regular distortions in the reconstructed strains maps.

On the contrary, a not evident result of the present study is that it is not recommended to use a fixed gradient-estimation scale $g_{x,z}$ (say, 2 or 3 pixels) in combination with the adaptive choice of sizes $W_{x,z}^{opt}$ for preliminary averaging as described above. In this case, it is likely that the so-found averaging-window size $W_{x,z}^{opt}$ might significantly exceed the chosen fixed gradient-estimation scale $g_{x,z}$. In this context, Eqs. (9) and (10) indicate that if the averaging-window size $W_{x,z}$ exceeds scale $g_{x,z}$, then the errors in the strain estimation may be even enhanced rather than reduced. Furthermore, the estimated strain acquires a regular bias that may be quite

significant (these effects are especially clearly seen in the right column in Fig. 6). However, these negative effects should not occur when adaptive optimization of the averaging-window sizes $W_{x,z}^{opt}$ is performed simultaneously with adaptive optimization of gradient-estimation scale $g_{x,z}$.

Overall, the presented analysis and results of processing of numerically simulated and real OCT records illustrate the efficiency of optimization of the averaging procedures in combination with optimization of the gradient-estimation scale for improving strain-mapping quality in phase-sensitive OCT elastography.

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Disclosures

The authors declare that they have no conflict of interest.

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