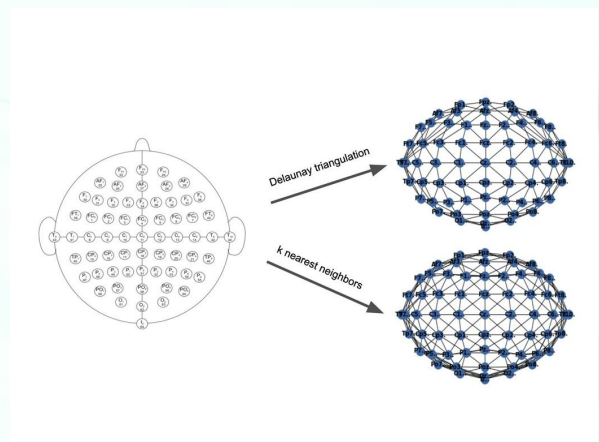
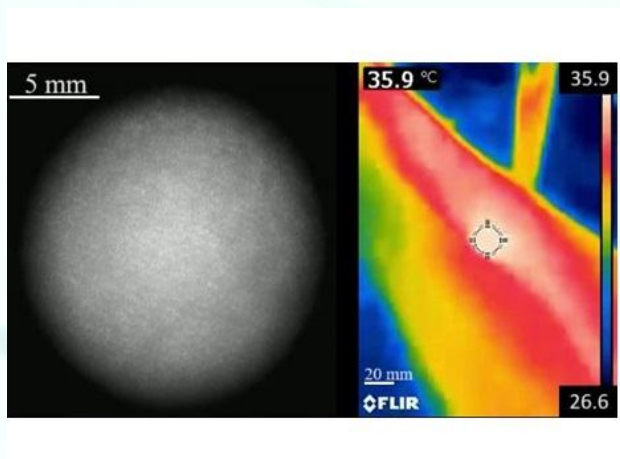

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Elina Genina and Ivan Bratchenko

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Valery Tuchin

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Special Issue

“Advanced Photonic Technologies in Biomedicine”

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Used Figures from this issue on the Cover

Andrey V. Belikov and Viktor Yu. Chuchin, Investigation of the Influence of Thermally Induced Methemoglobin on the Human Skin Optical Reflection, 020303, Fig. 4(a).

Leonid Sidorov and Archil Maysuradze, Enhancing Neurophysiological Analysis and Emotion Recognition with EEG Channel Spatial Metadata through Graph Neural Networks, 020304, Fig. 3.

Foreword to the Special Issue

Advanced Photonic Technologies in Biomedicine

We are pleased to present the special issue of JBPE, which focuses on photonic technologies for different biomedical applications. The issue contains selected papers presented at Saratov Fall Meeting 2023 (SFM-23) – XI International Symposium on Optics and Biophotonics (September 25–29, 2023, Saratov, Russia) and includes eight representative papers that well characterize the major topics of SFM-23.

The paper by Alexander A. Sovetsky et al. describes method for generation of realistic “digital phantoms”, which are OCT scans with fairly correct accounting for optical signal attenuation dominated by scattering. Authors present instructive examples demonstrating their usefulness for testing OAC-reconstruction approaches.

The paper by Andrey V. Belikov and Viktor Yu. Chuchin deals with the influence of thermally induced methemoglobin on the human skin optical reflection. The results obtained will allow one optimizing the parameters of laser for improvement of treatment of skin in dermatology and cosmetology.

Dmitry Artemyev and co-authors suggests the method of differential rapid diagnosis of endometrial cancer and its benign pathological conditions using surface-enhanced Raman spectroscopy (SERS) of blood plasma and machine learning. The approach presented has shown a high diagnostic efficiency.

Aleksey R. Krot and co-authors have studied self-assembled porphyrazine nanoparticles photosensitizers in aqueous solutions with and without a polyvinylpyrrolidone carrier. This work is the first to study a set of samples with a wide range of characteristics under passive load conditions.

The paper by Anna A. Doronkina et al. discusses the effects of upconversion nanoparticles on erythrocytes. Authors have shown that such interaction is accompanied by a change in cell shape, increased formation of aggregates, and affect both cell size and membrane viscosity. The results obtained are useful for the development of photodynamic therapy.

The study presented by Peter B. Ermolinskiy and co-authors demonstrates on the relationship between whole blood viscosity and microrheological parameters in the context of blood disorders.

Alexey A. Selifonov et al. have investigated optical properties of healthy and cancer ovarian tissue. The difference in the scattering and absorption properties of healthy and cancer ovaries can serve as

an important and promising marker of pathology in the early stages in clinical applications, as well as in the search and prove of diagnostically significant criteria for assessing the metabolic state of tissues.

In overall, papers collected in this special issue demonstrate well the exciting potential of optical technologies for biomedical studies and applications aiming medical diagnostics and treatment.

Special Issue Editors:

Elina A. Genina

Saratov State University, Tomsk State University, Russia

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Samara National Research University, Russia

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Enhanced Interaction of Ciprofloxacin with Humic Substances and Magnetite-Silica-Nanoparticles in Multicomponent System: Spectrophotometric and Electrokinetic Studies

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Abstract. We evaluated the effect of dissolved organic matter (DOM) on the sorption of antibiotic ciprofloxacin (CIP) by magnetite-silica nanoparticles (NPs). For this purpose, magnetite NPs (M) modified with organosilanes (tetraethoxysilane and 3-aminopropyltriethoxysilane, TA) were used as a natural model of colloidal particles and a sorbent. Absorption spectra did not manifest any interaction between CIP and humic preparation (HP, sodium salt of humic acids) in aquatic system at pH from 6 to 8. On the contrary, the introduction of the MTA NPs into a bicomponent system of CIP incubated with HP lead to more than twice increased CIP sorption. Results of electrokinetic measurements in terms of zeta potential figured out the interaction mechanism in the CIP-HP-NPs system. In the three-component system, the MTA surface completely recharged from negative to positive, and importantly this effect is pH-dependent. The MTA zeta potential varied from –15 (pH 6) and –30 mV (pH 8) to +10 (pH 6) and +2 (pH 8). The study provides novel information on very actual topic in biomedical and ecological research since NPs are increasingly produced and implemented for various medical and environmental applications, including sorption of emerging ecotoxins (pharmaceuticals), but the assessment of naturally occurring DOM in water should not be ignored. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: antibiotic; colloidal particles; natural anionic polyelectrolytes; dissolved organic matter; sorption; zeta potential.

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1 Introduction

The pharmaceuticals are currently increasingly contributing to environmental pollution [1]. Over the past decade, the production of antibiotics has doubled [2, 3]. Chronic use of antibiotics can pose a significant environmental risk due to improper disposal of expired and unused drugs [4]. Accidental or deliberate entry of antibiotics into wastewater is a common phenomenon that radically affects both chemical and physical properties of natural water and the ecosystem as a whole [5]. The ciprofloxacin (CIP), a fluoroquinolone antibiotic, is a synthetic broad-spectrum antimicrobial agent. Fluoroquinolones are not destroyed completely in the body, and as a result are released into the environment in large quantities. Their presence in the environment can cause the development and spread of drug resistance in bacteria [6], creating potentially serious threats to human health and the ecosystem. CIP is among the top ten antibiotic contaminants [7] in surface water and hospital wastewater, where CIP concentrations reach $6.3 \cdot 10^{-4} \text{ mg} \cdot \text{L}^{-1}$ and $0.1245 \text{ mg} \cdot \text{L}^{-1}$ respectively [8].

Various sustainable remediation technologies have been developed to remove CIP from water, including photocatalytic degradation, advanced oxidation processes (AOPs), biodegradation, electrochemical processes, membrane separation, and adsorption [9] using different classes of adsorbents such as carbon nanotubes (CNTs), carbon nanofibers, biochar, biosorbents, metal-organic frameworks (MOFs), and nanocomposites. Among the purification methods used according recent publications [9–10], adsorption treatment has proved to be effective due to numerous advantages such as low cost, ease of operation, simple design, reusability, insensitivity to CIP [11], energy safety [12]. In addition, no intermediate by-products are formed during this purification [13]. So many adsorbents and nanomaterials, including polymers, zeolites, activated carbon, and carbon-based adsorbents [9] such as magnetic N-doped nanoporous carbon [9], and mesoporous silica [14, 15] have been studied as potential adsorbents of CIP from aqueous solutions under different experimental conditions. For example, ordered carbon exhibited a notable CIP removal capacity of $233.4 \text{ mg} \cdot \text{g}^{-1}$ under specific conditions [16], activated carbon derived from bamboo demonstrated $362.9 \text{ mg} \cdot \text{g}^{-1}$ [16]. The potential of nanomaterials like graphene oxide/sodium alginate was highlighted by achieving a $100.00 \text{ mg} \cdot \text{g}^{-1}$ removal under particular circumstances [17]. Other materials, such as bentonite [7], cationic flaxnoil cellulose, and biochar from tea leaves, have also exhibited varying levels of CIP removal [9]. Carbon-based adsorbents, such as magnetic N-doped nanoporous carbon, exhibit higher q_{max} ($1563.7 \text{ mg} \cdot \text{g}^{-1}$) than other adsorbents. However, the efficacy of adsorption is heavily reliant on factors such as cost, the physicochemical properties of the adsorbent, solution pH, and competing solutes [9], underscoring the need for tailored adsorption systems to achieve efficient CIP removal. Among adsorbents proposed above, magnetite mesoporous silica nanomaterials are promising due to

economical reason, the large specific surface area and porosity (to $300 \text{ m}^2 \cdot \text{g}^{-1}$) [18], regular channel structure [19], inert, biocompatible [20] and reusable due to their very high saturation magnetization [21, 22]. In addition, due to the power of highly reactive hydroxyl radicals ($\bullet\text{OH}$) to degrade recalcitrant organic compounds in Fenton processes the AOPs using Fe_3O_4 NPs not only break down the CIP's chemical structure but also lead to the formation of potentially less harmful degradation products.

When assessing the potential of a sorbent should be taken into account the presence of dissolved organic matter (DOM) in real water. It will result in reducing sorbent efficiency for CIP. Humic substances (HS) are the main component of DOM [23] varying from $0.03 \text{ mg} \cdot \text{L}^{-1}$ for groundwater to $40 \text{ mg} \cdot \text{L}^{-1}$ for lakes [24]. Thanks to HS polyfunctionality, they strongly effect on the adsorption of any ecotoxicant onto colloid particles [25]. Thus, the present study is devoted to the evaluation of the effect of HS on the sorption of CIP in real environmental trace concentrations by magnetite silica-based sorbent. For this purpose, magnetite NPs (M) modified with organosilanes (tetraethoxysilane, T and 3-aminopropyltriethoxysilane, A) were used as a model of natural colloidal particles and a sorbent. In our previous research on MTA toxicity towards infusoria *Paramecium caudatum* [26] we demonstrated that the combined presence of the MTA-NPs at a concentration of $1.5\text{--}2 \text{ mg} \cdot \text{L}^{-1}$ and HA at a concentration of $20\text{--}45 \text{ mg} \cdot \text{L}^{-1}$ has a multiplier effect and allows increasing the survival rate of ciliates $> 70\%$ compared to MTA and HA alone due to the enhanced removal of CIP.

From biomedical point of view, the produced nanosystems based on mesoporous silica including magnetic-based can be considered as a potential system to be applied in bacterial infection treatment [18], for the diagnosis and therapy of various diseases such as in tumor therapy [27] and as contrast agents for bioimaging [28, 29], nanocarrier [30] for drug delivery [31], including for targeted ciprofloxacin [31]. A simple and inexpensive dispersive solid phase extraction method was used by Nosratsehi et al. [32] based on $\text{SiO}_2 @ \text{MCM-41-Co}_3\text{O}_4$ yolk shell as a sorbent for the extraction of favipiravir (5-fluoro-2-oxo-1 H-pyrazine-3-carboxamide, first used to treat Covid 19 disease) from plasma samples. Porphyrinoids coated silica nanoparticles was tested as capacitive sensors for COVID-19 detection from the analysis of blood serum volatolome [33]; niclosamide encapsulated in mesoporous silica and geopolymer was proved to be a potential oral formulation for COVID-19 (Niclosamide encapsulated in mesoporous silica and geopolymer: A potential oral formulation for COVID-19). The presence of polyanionic polyelectrolyte (HS) can be considered as promising chemisensitizing agents in therapeutic alternatives for any biopreparations due to their detoxification, anti-, and pro-inflammatory or anticancer and antiviral ability [34, 35].

2 Materials and Methods

2.1 Synthesis of Magnetite NPs

The bare magnetite nanoparticles (MNPs) were prepared by the coprecipitation method described in Ref. [36]. Briefly, 7.56 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Joint Stock Company “Bromine”, Russia) and 2.78 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Joint Stock Company “Altayskiy Chimprom”, Russia) were dissolved in 70 mL of H_2O , then 40 mL of 25% solution of NH_4OH was added at 50 °C under argon flow and vigorous stirring at 1600 rpm during 20 min. The NPs prepared were washed three times with deionized water and twice with alcohol to remove the synthesis residues then dried at 70 °C under vacuum, yielding 2.9 g of the product.

2.2 Synthesis of Magnetite-Silica NPs (MTA)

The synthesis of silica-coated magnetite NPs was carried out by the well-known Stöber method [37]. The previously synthesized MNPs were used as a core to be coated with TA. MNPs weighing 5.8 g were suspended in 300 mL of DI water. To 25 mL of tetraethoxysilane (hereinafter referred to as T, 98% purity, Sigma-Aldrich), 225 mL of H_2O and 350 mL of $\text{C}_2\text{H}_5\text{OH}$ were added with constant stirring at 600 rpm, and finally, a suspension of MNPs was added to the resulting solution. The pH of the suspension MT was adjusted to 9 by 5 mL of 25% of NaOH and placed on a shaker (New Brunswick Environmental incubator shaker G24) for 1 h at 150 rpm and 60 °C. The MT precipitate was separated from the solution using an Nd magnet (0.3 T) and washed with 300 mL of H_2O to remove excess T, then twice with alcohol for drying. The 3-aminopropyltriethoxysilane (further A, 98% purity, Sigma-Aldrich) was used as a NH_2 -silica precursor. MT NPs weighing 3.2 g were dissolved in 150 mL of a DI water/ethanol solution (1:1 vol/vol), and then 6.2 mL of A was added to the solution in an argon atmosphere at 40 °C for 2 h according to Ref. [38]. Molar ratio of A to T was used as a 1:4. The precipitate was isolated with a magnet (Nd, 0.3 T) and thoroughly washed with water and alcohol before analysis or further use. Finally, the MTA sample was vacuum-dried at 70 °C during 2 h, yielding 6.9 g of the product.

2.3 Characterization of Samples

The structure of the surface layer of NPs may have profound effect on the toxicity of NPs as a surface is the first to be contacted with the cells [26]. Therefore, X-ray diffraction analysis (XRD) method was used to investigate the phase composition and estimate the average particle size of samples using a Philips X-pert diffractometer (Philips Analytical, Eindhoven, The Netherlands, $\text{Cr-K}\alpha$ radiation, $\lambda = 2.29106 \text{ \AA}$) as revealed in Ref. [39]. Average particle size was determined with the Scherrer Equation using the full width at half maximum (FWHM) of all reflections in the Origin 2019 Pro software.

The lattice parameters established for M and MTA NPs were below than that of magnetite Fe_3O_4 as 8.396–8.400 Å (ICDD-PDF 19-629) but above than that of maghemite $\gamma\text{-Fe}_2\text{O}_3$ as 8.33–8.34 Å (ICDD-PDF 39-1346). The formation of non-stoichiometric $\text{Fe}_{3-\delta}\text{O}_4$, where δ can range from 1 (stoichiometric magnetite) to 1/3 (fully oxidized) [40] due to partial oxidation of Fe^{2+} during synthesis conditions.

The morphology of the NPs was investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Prior to SEM imaging, the samples were sputter coated with a thin film (approximately 10 nm) of a gold-palladium (Au-Pd) alloy using a Bal-Tec SCD500 coater. The morphology of the sputter-coated samples was examined using a Sigma 500 VP FE-SEM (Carl Zeiss, Oberkochen, Germany) with an accelerating voltage of 3 kV.

For TEM analysis, the NPs were dispersed in deionized water using an ultrasonic processor (Fisherbrand, USA) set at 75% amplitude to facilitate homogenization and prevent particle aggregation. A 10 μL aliquot of the suspension was applied to a 200-mesh carbon-coated copper grid (Ted Pella Inc., USA) pretreated with a glow discharge cleaning system (PELCO Inc., USA) to enhance sample adhesion. After one-minute incubation at room temperature, excess liquid was carefully removed with filter paper. The prepared grids were then mounted on a TEM stage for further analysis. High-resolution TEM images were obtained using a Tecnai G2-20 transmission electron microscope (FEI Company, Hillsboro, OR, USA) with an accelerating voltage of 120 kV. Particle size distribution was determined by analyzing at least 100 particles per image using ImageJ software (National Institutes of Health, USA).

Mechanism of modification was proved using the Fourier transform infrared spectroscopy (FTIR) analysis in the range of 4000–400 cm^{-1} on a FTIR spectrometer (FTIR IR-200, ThermoNicolet, USA). The samples were pressed into pellets $\varnothing = 13 \text{ mm}$ with spectrally pure KBr.

The specific surface area and characteristics of the porous structure of samples were determined using N_2 adsorption-desorption analysis (Quantochrome). To prepare the N_2 adsorption isotherms, the volume of gas-adsorbate (N_2) sorbed by the surface of the samples studied was measured when a stationary flow of a gas He-N_2 mixture of a given composition was passed through them (the volume fraction of N_2 varies from 0 to ≈ 1) at a temperature liquid N_2 (77 K) and desorbed by heating (up to approximately 100 °C). Similarly, for desorption isotherms, the volume of N_2 desorbed from the surface of the studied samples was measured with a decrease in its volume fraction in the gas mixture from ≈ 1 to 0. The specific surface area and characteristics of the porous structure were calculated based on the adsorption-desorption isotherms using the Brunauer-Emmett-Teller (BET) and Barret-Joyner-Halenda (BJH) methods. Prior the measurement, the test samples were degassed and vaporized, and “thermally trained” for

30 min by heating in a stationary N₂ flow in a vacuum at 150 °C.

Particle surface charge (zeta-potential) was analysed using electrophoretic light scattering on Brookhaven apparatus at a wavelength of 633 nm with a solid-state He-Ne laser at a scattering angle of 173° at 25 °C. For the analysis, each sample was diluted in water to 0.1 g·L⁻¹. Before the analysis the samples were ultrasonicated (ultrasonic bath, 100 W, 40 kHz) for 10 s of ultrasonication followed by 100 s of standstill. Samples were analysed in a disposable zeta cuvette (DTS 1070) at their original pH (as in synthesis, ranging from 5.4 to 7.5). The pH values were measured prior and after the study.

2.4 Equilibrium Sorption Studies

A sorption technique was used to quantify CIP sorption onto the NPs in the presence of humic preparation (HP, sodium salt of humic acids, Powerhumus, Humintech, Germany; 5 mmol·g⁻¹ of COOH and OH-groups, Mw = 8 kD). For the adsorption experiments, 1 and 2 g·L⁻¹ of NPs and/or 0.01 and 0.05 g·L⁻¹ of HP were weighed and placed into a 200 mL conical flask and filled with 100 mL of water with CIP concentration of 5 mg·L⁻¹. All flasks were rotated at 150 rpm in the dark on a rotary shaker for 24 h at 23 °C. After the reaction time of 24 h, the NPs and the supernatant were separated as described above. At the end of the equilibration period, the suspensions were centrifuged at 1000 rpm for 10 min and the supernatants were extracted by syringe and filtered through a hydrophilic membrane (0.45 µm) for CIP determination by UV-Vis spectroscopy. After filtration, the pH of the supernatant was measured (Supplementary Materials, Table S1). Equilibrium CIP concentrations were determined by transferring 2 mL aliquots to UV-Vis cuvette. Samples were analyzed on a Solar UV-Vis spectroscopic system equipped with a fluorescence detector.

To determine the concentration of CIP in the presence of HP the spectrophotometric measurements, the CIP solutions and HP were prepared in different concentrations in water. The CIP concentration in the solution varied from 1 to 20 mg·L⁻¹, the HP concentration was stepwise increased up to 50 mg·L⁻¹. In addition, the influence of the pH on the spectral characteristics was studied with pH altering from 5 to 8. When preparing solutions of CIP in the presence of HP, the pH values levelled off for all samples at about 7.0).

The absorption spectra were registered using a Solar PB2201 spectrophotometer (Solar, Belarus) at room temperature. The measurements were performed in the spectral range 200–600 nm with 1 nm interval using standard quartz cuvettes with an optical path length of 10, 5, or 2 mm depending on the absorbance of the studied samples. After measurements all absorbances were normalized to 1 cm pathlength.

3 Results

3.1 Characterization of Samples (XRD, IR, BET Analysis)

The XRD was used for the analysis of phase composition and primary particle size of the samples. The M and MTA samples are well crystallized materials according to intense and broadened diffraction reflections (Fig. 1) with Miller indices of (220, ~45°), (311, ~54°), (400, ~66°), (422, ~84°), (511, ~91°), and (440, ~101°). According to the quantitative analysis carried out by the Rietveld method, all diffraction patterns of the samples can be attributed to samples with a face-centered cubic (Fd3m) lattice and, ultimately, to partially oxidized magnetite. The functionalization of TA during the sol-gel synthesis leads to a decrease in the concentration of stiochiometric magnetite. So, for bare magnetite the crystal cell parameter is 8.383 Å, while for TA functionalized NPs that made 8.364 Å.

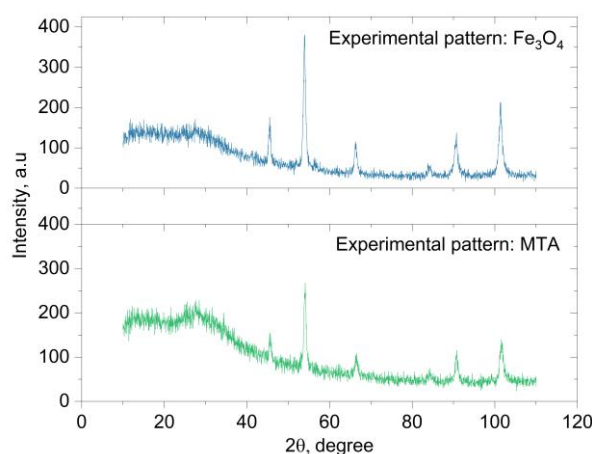


Fig. 1 XRD for M and MTA NPs samples.

The functionalization of TA leads to a slight change in the size of the coherent scattering region from 21.9 nm to 18.8 nm, which was calculated according to the XRD data using the Scherrer Equation. These values are in good agreement with the literature data [41, 42]. Moreover, according to transmission electron microscopy data, MNPs embedded in the TA matrix have an average size of 14 ± 3 nm and have a shape close to spherical (Supplementary materials, Fig. S6). This characteristic size and morphology indicates superparamagnetic nanoparticles behaviour. Scanning electron microscopy analysis of the MTA composite showed the presence of aggregated particles measuring 88 ± 15 nm with an ill-defined morphology (Supplementary materials, Fig. S6).

Analysis of the specific surface area (SSA) of the MTA NPs by the BET method and the average pore diameter by the BJH method found that the silica modification of M increase all surface characteristics for the MTA including the specific surface area, pore volume, and micropore volume (Table 1).

Table 1 Textural characteristics of samples.

Parameter	M	MTA
Pore volume, $\text{cm}^3 \cdot \text{g}^{-1}$	0.20 ± 0.01	0.96 ± 0.03
Pore width, nm	15.8 ± 0.9	32.7 ± 1.3
SSA MultiPoint BET, $\text{m}^2 \cdot \text{g}^{-1}$	63.8 ± 3.8	119.6 ± 5.4

The observed increase in the textural characteristics of magnetite-silica NPs is consistent with the results of studies [43].

Desorption hysteresis loops are observed on N_2 adsorption-desorption isotherms of the studied samples (Fig. 2). According to the BET classification in terms of adsorption isotherms shape (type IV) and to the International Union of Pure and Applied Chemistry (IUPAC) in terms of hysteresis loop shape (type H3), both samples have a mesoporous structure with a slit-like pore shape.

The mechanism of stabilization and functionalization of MNPs with TA was studied using FTIR spectroscopy. Si-OH silanol groups on the silica surface make it easy to organize the covalent attachment of ligands to the surface of M. To functionalize the TA surface, we used the sol-gel synthesis of NPs consisting of *in situ* hydrolysis and condensation of the sol-gel precursor (Fig. 3).

At the first stage of silica surface modification using TA, alkoxysilyl groups are hydrolysed with the formation of strongly grouped Si-OH. Groups of modifiers react with OH groups on the surface of silicon dioxide with the release of water and the formation of an anchor bond Si-O-Si-C. The introduction of A on the surface of MT NPs in our case is necessary to create terminal amino groups, which are subsequently connected in combination with commonly available classes of compounds in order to obtain functional and hybrid materials.

M is able to sorb SiO_2 due to the presence of OH-groups on the surface of iron oxide, which leads to the appearance of the Fe-O-Si bond and is confirmed by the appearance of transmission bands in the spectrum for MTA at wavelengths of 692 cm^{-1} , 583 cm^{-1} . The formation of the Si-O-Si bond characteristic of the silicon framework as a result of the sol-gel synthesis is confirmed by the appearance of a band at 1056 cm^{-1} , and the presence of C-H bonds indicates incomplete polymerization.

Comparison of IR spectra obtained with Fourier transform and Attenuated total reflection (ATR) spectra shows that ~12% of the Si-O-Si bonds are on the MTA surface. Comparison of the ATR IR and FTIR spectra shows that the free -NH_2 (790 cm^{-1} , 1633 cm^{-1} , 3424 cm^{-1}) or OH- groups (1633 cm^{-1} , 3424 cm^{-1}) localized partly on the NPs surface.

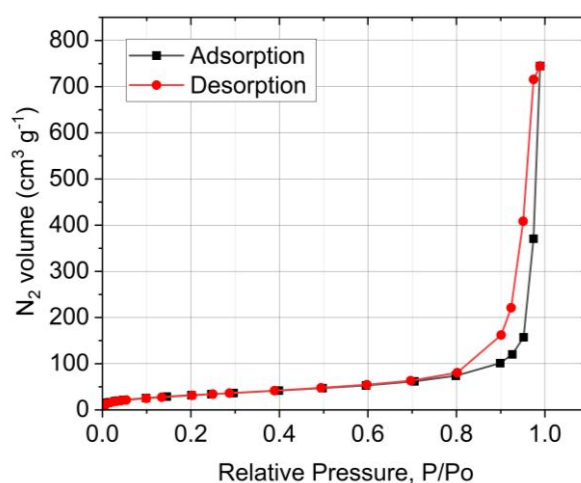


Fig. 2 Isotherms of N_2 adsorption and desorption of MTA.

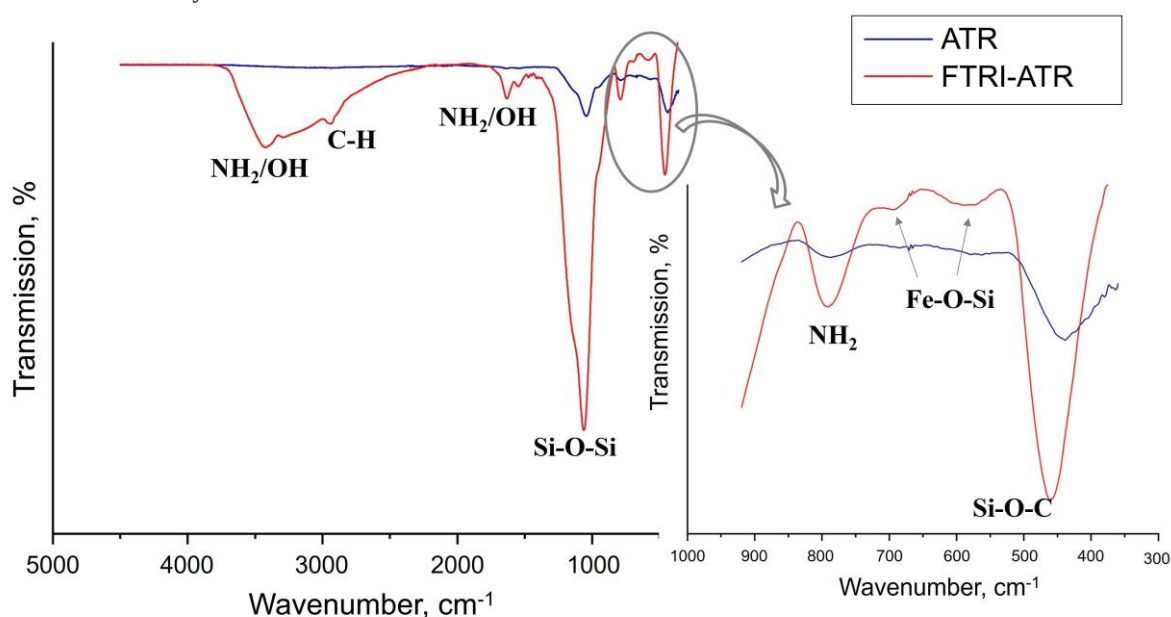


Fig. 3 IR-spectra of MTA: FTIR and ATR analysis.

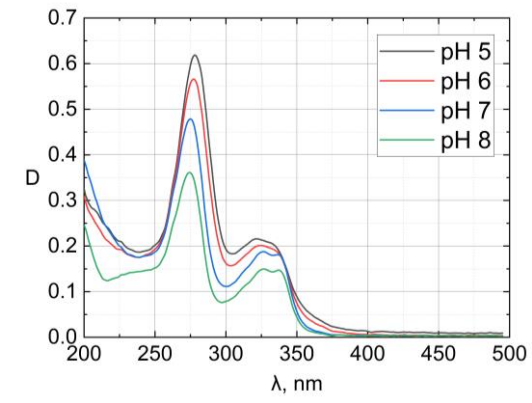


Fig. 4 UV-Vis spectra of a CIP solution in water at a fixed concentration $5\text{ mg}\cdot\text{L}^{-1}$ and different pH values.

3.2 Sorption Experiments: Spectrophotometric Measurements

Optical methods based on the measurement of absorption spectra make it possible to expressly analyse water pollutants by the absorption bands of the pollutant [44]. In the case of CIP, the UV absorption bands with maxima at 270–280 nm and 320–330 nm can be used. However, an obstacle in determining the concentration of CIP in natural or technological water is the presence of HS occurring in natural or waste water. The HS have high absorbance in the UV range and monotonically decreasing light absorption towards longer wavelengths, almost disappearing at about 500 nm [45, 46]. The proposed in this work method of CIP quantification in water in presence of HP was applied to determine the antibiotic concentration in an aqueous medium after its partial removal by various sorbents.

3.2.1 Absorption Spectra of CIP in Water at Different pH Values

When considering the CIP absorption spectra, one could notice the UV-band with a higher intensity at $\lambda_{\text{max}} = 270\text{--}280\text{ nm}$, and the second UV-band with a lower intensity at $\lambda_{\text{max}} = 320\text{--}330\text{ nm}$. Upon changing the pH of the medium at a constant CIP concentration, the following changes in the absorption band occurred: the

shift of maximum position and the change in absorbance value. The intensity of the UV-bands decreased with a change in pH from 5 to 8 (Fig. 4).

To avoid the problem with alteration of maximum position with pH we suggest to quantify absorption intensity not by the absorbance value D taken at certain wavelength, but by the area under the absorption curve (S) in the wavelength range from 260 to 300 nm. The calculation confirmed the dependence of the absorption intensity on the medium acidity: in solution with pH 5–6, the CIP absorbance turned out to be approximately 30% higher than in solutions with pH 7–8.

The dependence of the area S of the antibiotic absorption on the CIP concentration C was plotted (Fig. S1, see Supplementary Materials). This dependence turned out to be proportional, the coefficients of proportionality (k) are given in Table 2 for the studied pH values. The proportionality of the dependence allows it to be used to determine the CIP concentration in water at different pH values. The general formula for determining the concentration C will be as follows: $C = K \cdot S$, where $K = 1 \cdot k^{-1}$ is an empirical coefficient depending on the pH of the medium. The K values are shown in Table 2 for the studied pH values of the aqueous solution. Thus, it is possible to obtain a calibration dependence of the absorption band area of CIP on concentration to determine the antibiotic concentration in water with known pH.

Table 2 Values of coefficients k and K for different pH values of an aqueous solution.

pH	$k, \text{nm}\cdot\text{L}\cdot\text{mg}^{-1}$	$K, \text{mg}\cdot(\text{L}\cdot\text{nm})^{-1}$
5	3.40	0.29
6	3.37	0.30
7	2.53	0.40
8	2.25	0.44

3.2.2 Absorption Spectra of HP and Mixtures of CIP and HP in Water

The UV-Vis spectra of the HP solution in water is a monotonically decreasing curve, the intensity of which decreases in the direction of increasing wavelength (Fig. 5).

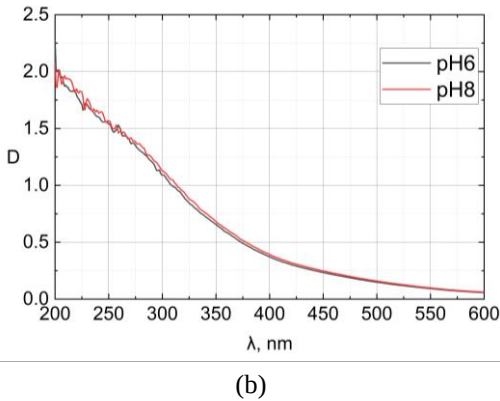
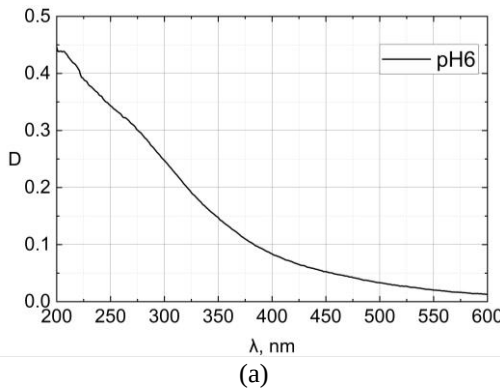


Fig. 5 (a) UV-Vis spectra of a HP ($10\text{ mg}\cdot\text{L}^{-1}$) at pH 6; (b) Absorption spectrum of a HP ($50\text{ mg}\cdot\text{L}^{-1}$) at different pH.

The absorbance value at each wavelength is almost independent on the pH of the medium. The absorption spectrum of the (CIP+HP) mixture is shown in Figs. S2 and S3 (Supplementary Materials).

HS are soluble in neutral and alkaline media [47]. This property varies depending on the chemical composition and origin of the HS. In an alkaline medium, phenolic and carboxyl groups are deprotonated (pK_i are approximately 4 and 9), negatively charged groups are repelled, and HP molecule acquires linear configuration. As the pH shifts to the acidic region, the functional groups of HS are protonated, the repulsive effects are minimized, and the molecules acquire a helical conformation, in which the hydrophobic regions are located in the inner part of the structure, and the hydrophilic parts are in contact with the aqueous medium. In this case, an increase in the concentration of HS can lead to the inaccessibility of reactive groups and steric hindrances in the CIP sorption. Fang et al. [48] noted that dissolved HP can act as an electron shuttle to accelerate electron transfer and CIP degradation.

The algorithm with the subtraction of the absorption band of the HP was repeated for all concentrations of the antibiotic in solution studied: 5, 10, 15, and 20 $\text{mg}\cdot\text{L}^{-1}$ (Fig. 6).

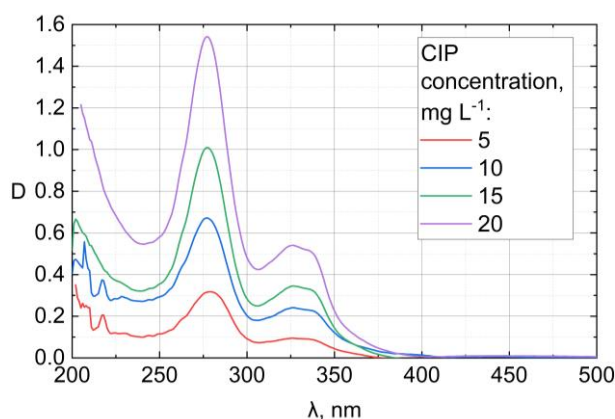


Fig. 6 UV-Vis spectra of CIP at different concentrations in water with the addition of HP at a concentration of 50 $\text{mg}\cdot\text{L}^{-1}$. Absorption of HP was subtracted from the spectrum of the (CIP + HP).

3.2.3 Calibration Curves for CIP

Determination in Water in Presence of HP

As a result, the dependence of the CIP absorption band area S on the CIP concentration obtained from the absorption spectrum of a multicomponent solution by subtracting the HP absorption band was plotted. This dependence also turned out to be proportional with slopes close to those obtained earlier for aqueous solutions of the CIP ($\text{pH}=8$) without HP (see Table 2). The similarity of the shape of the CIP absorption bands in the absence and with the addition of a HP (compare Figs. 6 and 7) led to the conclusion that there are no noticeable interactions between HP and antibiotic.

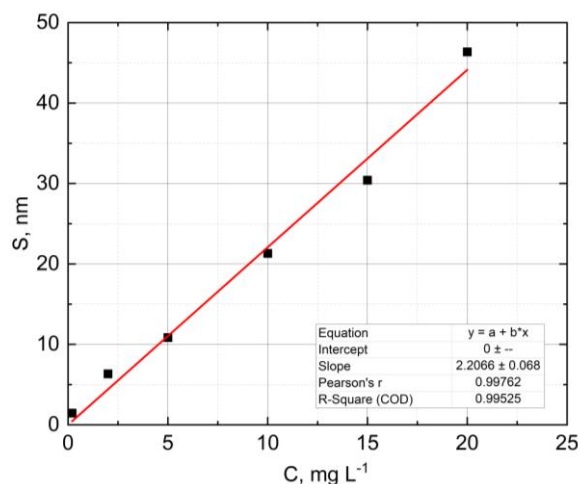


Fig. 7 Dependence of the area of the absorption band of CIP when the spectrum (HP) is subtracted from the spectrum (CIP+HP) on its concentration in the sample.

Thus, even in the presence of HP, the same formula can be used to determine the concentration of the antibiotic CIP in the aquatic environment: $C = K \cdot S$, where $K = 1 \cdot k^{-1}$ is an empirical coefficient determined as an average value according to Table 3.

Table 3 The coefficients k and K for the mixtures of HP and CIP.

Sample	k , $\text{nm}\cdot\text{L}\cdot\text{mg}^{-1}$	K , $\text{mg}\cdot(\text{L}\cdot\text{nm})^{-1}$
CIP (concentration 5, 10, 15, or 20 $\text{mg}\cdot\text{L}^{-1}$) + HP (50 $\text{mg}\cdot\text{L}^{-1}$)	2.21	0.45

3.2.4 Absorption Spectra of CIP in Water in the Presence of HP and MTA

The study of sorption properties of MTA in the presence of CIP showed that the addition of HP to a solution with pH 6 and 8 leads to pH equalization to ~ 7 . The introduction of the MTA NPs as a sorbent into a system with CIP incubated with HP leads to a 2-fold increase in the sorption capacity (Fig. 8).

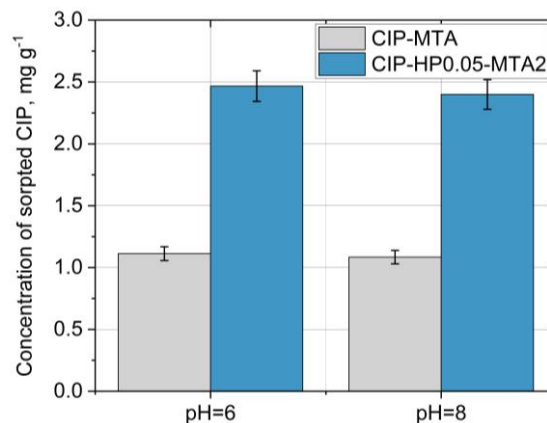


Fig. 8 Concentration of CIP sorted by MTA with and without of HP at different pH.

3.3 Proposed Mechanism of Sorption CIP by MTA and HP: Zeta Potential Measurements

To understand the mechanism of CIP sorption by NPs at various pH, the surface charge of NPs in terms of zeta potential was analysed using the dynamic and electrophoretic light scattering method. Dynamic light scattering is one of the most commonly used photonic methods for estimating the hydrodynamic radius, ζ -potential, polydispersity of nanoparticles, as well as for studying changes in these parameters upon interaction or aggregation of molecules and particles [49]. CIP is a zwitter-ionic compound. Below pH 5.9 ($pK_{a1}=5.9$), the predominant CIP species are cations. The surface of MTA is presented as positively charged, which causes an electrostatic repulsion between the available cationic species CIP and MTA.

The pH value is a key parameter that is critical for the removal of CIP molecules from aqueous systems because it affects adsorbate ionization, ion competition, and deprotonation of sorbent functional groups. The study of the dependence of the MTA zeta potential on pH showed (Fig. 9) that the isoelectric point of the NPs is at pH 5.5 (pH_{IEP}), which indicates the presence of a small amount of free amino groups on the surface. In the range $pH < 4$ and $pH > 6.5$, the zeta potential is greater than ± 25 mV, which indicates the stability of the sample due to electrostatic repulsive forces.

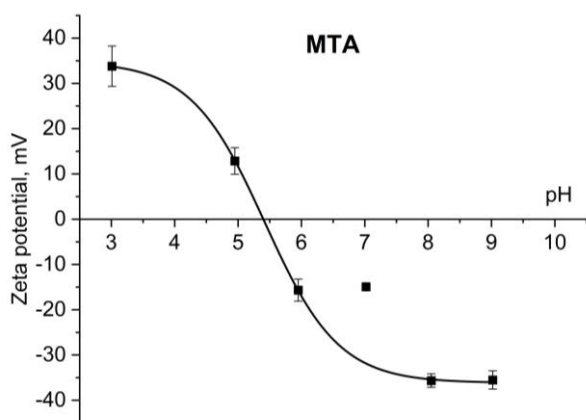


Fig. 9 The function of zeta potential of NPs of pH.

According to Refs. [50, 51], CIP carbonyl groups are deprotonated at 5.9 (pK_{a1}), and amino groups are deprotonated at 8.9 (pK_{a2}), which, for example, differs from the data of Wu et al. [52] reporting 6.1 and 8.7. After all, CIP can have different surface charges and exist as a cation, anion, and zwitterion [53], as shown in Fig. S5. With a total negative surface charge, absorption occurs through cation exchange with the protonated amino group. With a net positive surface charge, sorption occurs due to complex interactions of deprotonated carboxyl groups [54]. This is where the adsorbent IEP (pH_{IEP}) comes into play.

Below pH 5.9 ($pK_{a1} = 5.9$), the predominant CIP species are cations; under these conditions there is a high

concentration of the H^+ ions with smaller ionic radii and a higher adsorption capacity, which hinders the adsorption of CIP molecules creating competition between excess protons in solution and cationic CIP molecules (protonation of $-NH_2$ groups). Also, according to electrophoretic light scattering data, at $pH < pH_{IEP}$ (5.5), the MTA surface appears to be positively charged, which causes an electrostatic repulsion between the available cationic species CIP and MTA (Fig. 10).

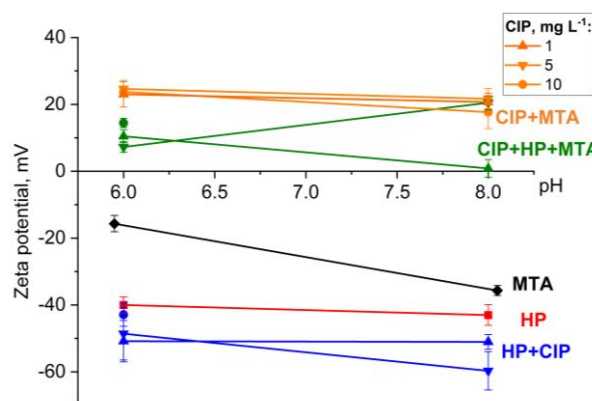


Fig. 10 Effect of HP and CIP adsorption on the surface charge of MTA NPs at different pH (0.05 $g \cdot L^{-1}$ of HP and 2 $g \cdot L^{-1}$ of MTA).

Thus, lower CIP sorption at lower pH values can be attributed to competitive sorption and saturation of adsorption sites on the MTA sorbent surface with hydrogen ions along with repulsive forces between CIP molecules and the protonated surface [55]. When the pH shifts to the alkaline region, the competition between protons and antibiotic molecules decreases and leads to an increase in the percentage of removal. If we talk about the mechanism, then an increase in pH leads to the interaction of CIP molecules with the functional groups of the sorbent (amino and hydroxyl groups) by ion-exchange mechanisms, which leads to an increase in the negative surface charge and electrostatic attraction, and, ultimately, to a higher efficiency of removal of CIP.

In addition, in the pH range ($pK_{a1} = 5.9-8.9$), CIP molecules are in an insoluble state and have a higher hydrophobicity, which leads to a hydrophobic interaction between the adsorbent/adsorbate and an increase in adsorption efficiency [55]. Moreover, at $pH > pK_{a2}$, a tendency to a decrease in the removal efficiency was recorded, which could be due to the electrostatic repulsive interaction between the negatively charged MTA surface (deprotonation of $-OH$ groups) and CIP molecules, as well as due to the saturation of MTA binding sites with CIP molecules. The recharging of the sorbent surface during the sorption of CIP from negative to positive may be associated with nonequivalent adsorption of the antibiotic on the NPs surface (an excess of antibiotic).

Thus, based on the electrostatic mechanism, it can be assumed that the higher sorption of CIP on the MTA

surface will be observed in the range of pH 5.9–8.9, where the negatively charged surface of the sorbent and the positively charged amino groups of CIP will be attracted. HA have a negative charge over the entire range of pH [56]. At the same time, the addition of CIP does not lead to a significant change in the zeta potential: the zeta potential value increases from -40 mV to -50 mV, probably due to sorption or the presence of CIP in solution, which can be adsorbed on the surface of HP due to positively charged amino acids groups.

At the same time, the addition of HP to the MTA-CIP complex leads to a decrease in the positive charge due to the dominance of negatively charged groups [57] on the HP surface compared to the CIP-MTA surface. The concentration of CIP does not lead to a change in the charge of MTA and HP.

4 Conclusions

The absorption properties of nanoparticles towards widely used antibiotic ciprofloxacin (CIP) in the presence of humic preparation (HP) as a model of dissolved organic matter (DOM) were analysed using spectrophotometric and electrokinetic studies. In absorption spectral measurements, the proportional dependence of the CIP absorbances and its concentration was established, and it was noticed that the absorption maximum shifts to a shorter wavelength range (278–274 nm) with pH increased from 5 to 8. Therefore, we suggested to use as a quantitative parameter of CIP concentration the area under the UV bands in the CIP absorption spectra. In presence of HP we subtracted the shapeless absorption spectrum of the HP to get CIP absorbances, which coincided with the previously obtained spectral absorbances for pure antibiotic. The fact that the absorption spectra of a multicomponent solution of CIP and a HP are a superposition of their individual absorption spectra indicates the absence of noticeable interactions occurring between the CIP and HP.

Analysis of the absorption spectra of CIP in the presence of silica-based magnetic NPs at different pH showed the influence of pH and HP in the CIP-MTA system. Thus, the sorption of CIP NPs increases by a factor of 1.5 when pH changes from 6 to 5. The introduction of NPs into a system with CIP incubated with HP leads to an increase in the sorption capacity by more than 2 times.

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Based on electrokinetic measurements, the mechanism of interaction in the CIP-HP-MTA system was proposed. Thus, in a three-component system, the surface is completely recharged from negative to positive: the MTA surface charge changes from -15 (pH 6) and -30 mV (pH 8) to $+10$ (pH 6) and $+2$ (pH 8), respectively. With an increase in pH from 6 to 8, CIP approaches the zwitterionic form, while the adsorption capacity of the formed MTA NPs increases, since the interaction of neutral CIP with the negatively charged surface of the obtained MTA NPs became more possible, but CIP is less soluble. At pH above 8.8, both CIP and the formed MTA NPs were negatively charged, which limited the interaction of CIP with the surface of the formed MTA NPs.

The study provides novel scientific information as NPs are increasingly produced and studied for various biomedical and environmental applications, including sorption of new emerging ecotoxins (pharmaceuticals), however the assessment of DOM contained in natural water even in trace concentrations should not be ignored. A systematic investigation will be a subject of future study of the adsorption performance towards CIP, by varying the initial pH, contact time and initial CIP concentration.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Simple “Digital Phantom” for Testing Attenuation-Imaging Methods in Optical Coherence Tomography

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Abstract. The manuscript describes a simple but realistic and efficient method for generation of OCT scans with fairly correct accounting for optical signal attenuation dominated by scattering. The strong domination of scattering in the optical attenuation coefficient (OAC) is typical of OCT where the illuminating-beam wavelength is intentionally chosen in optical transparently windows with minimal absorption. At the same time the scattering-dominated OAC may strongly differ for various types/states of biological tissues, so that the diagnostic value of OCT examinations can be significantly increased due to spatially-resolved estimation of OAC. However, the results of OAC-reconstruction may be strongly degraded by the “speckle noise” intrinsic to OCT scans, various measurement noises, and some other factors. Verification of OAC-reconstruction accuracy in physical experiments is challenging since controllable variation in the parameters of physical phantoms and OCT systems is often difficult or even impossible. In view of this, realistic numerical simulations may open unprecedented possibilities for testing and comparison of various OAC methods in highly controllable and flexibly variable conditions. Here, we describe a method enabling generation of realistic “digital phantoms” and present instructive examples demonstrating their usefulness for testing OAC-reconstruction approaches. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: optical coherence tomography; OCT-scan formation modeling; speckle pattern; optical attenuation imaging.

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1 Introduction

Development of new modalities beyond conventional structural imaging has become one of the main trends in Optical Coherence Tomography (OCT) [1]. In particular, this concerns such actively developing modalities as Optical Coherence Angiography in various forms [2–4], as well as various approaches to realization of Optical Coherence Elastography [5–7]. Also diagnostic approaches are known which are based on deep analysis of speckle-pattern parameters in OCT (e.g. [8, 9]).

Another active direction is the development of approaches aimed at assessment of optical attenuation coefficient (OAC) based on the analysis of signal decay

in OCT scans. Various methods of OAC visualization are discussed in several recent reviews, e.g. [10, 11]. Although optical-signal attenuation for forward propagation is described by a simple exponential factor, for estimation of the attenuation coefficient by analyzing OCT scans, the situation is less trivial. For formation of OCT scans, besides attenuation in the medium, there are other factors that also affect the signal amplitude (in particular, there is such an evident factor as the influence of OCT-beam focusing, as well as the so-called roll-off effect intrinsic to the most widely spread spectral-domain OCT devices). Furthermore, even if these factors are eliminated or corrected, the depth dependence of signal decay in OCT scans may be exponential only for spatially

uniform attenuation coefficient in the studied medium. At the same time, for diagnostic purposes in biomedical applications of OCT, the most interesting is spatially-resolved mapping of the spatially-inhomogeneous attenuation rather than obtaining of only spatially-averaged estimates assuming the decay described by a single exponential function. It can also be pointed out that methods of spatially-resolved OAC estimation are interesting not only for differentiation of various types of biotissues (e.g. cancerous tissues from normal ones which may differ by the OAC values [12]). Namely, one of active fields in biophotonics is the development of technologies of artificial reduction of OAC values for biological tissues, also termed optical clearing methods in studying of which pioneering results were obtained by the research group of professor Valery Tuchin from Saratov State University [13, 14]. For optical clearing, tissues usually are impregnated with a clearing agent which gradually diffuses into the tissue bulk from the surface leading to gradual, initially spatially inhomogeneous reduction of OAC, so that the possibility to perform monitoring of OAC variations with one-side OCT examination represents an evident interest.

In view of this, in the recent decade, significant attention has been paid to the development of OCT-based methods allowing for spatially-resolved attenuation imaging. An important step in solving this problem was the work by Vermeer et al. [15], where an approach to spatially-resolved OAC estimation from OCT scans was proposed for the case of strong domination of contribution of scattering to the OCT-signal decay. This assumption about the scattering dominance is usually quite reasonable; however, there are some other factors that may significantly worsen the operability of method [15]. For example, among these factors are incomplete decay of the OCT signal within the visualized depth or the influence of various noises, including the reception noise, as well as the “speckle noise” intrinsic to OCT scans. Consequently, there are quite many studies devoted to mitigation of the above-mentioned negative factors in approach [15], for example [16, 17]. Certainly, other OCT-based methods proposed for OAC estimation using are also susceptible to various degrading factors.

For verification of operability and accuracy of various OCT modalities quite often various experimental tests on specially prepared phantoms with *a priori* known properties are used. Phantom-based tests were used for verification various OCT-based diagnostic methods, for example, for determining the density of scattering centers based on estimation of speckle contrast [18], for OCT-based flow-velocity estimation [19], as well as in the context of OCT-based OAC estimation [15]. Properly prepared phantoms were considered for imitating various properties of biotissues, including mechanical, optical, and structural ones [20]. However, creation of sufficiently controllable properties of physical phantoms is a rather difficult problem. Furthermore, maybe even more difficult is to provide in physical experiments the possibility of flexible variation of experimental

parameters, which concerns parameters of both the imitated tissues and the used OCT-systems.

In this regard, from the first years OCT development till present, significant attention is paid to creation of convenient and at the same time sufficiently realistic models describing formation of OCT signals. Using such models various OCT-based methods can be tested. In particular, much attention was paid to modeling speckles in OCT scans [21]. For example, such modeling was helpful for understanding the degrading role of OCT speckles for quality of structural OCT images [21], or for understanding limitations of speckle tracking in OCT-based elastography [22]. Numerous other studies were focused of the diagnostic value of assessment of OCT-speckle characteristics rather than on their degrading role. For example, along with earlier models like work [21] based on summation of several phasors with random phases in individual pixels or more complex heuristic models (e.g. [23]), a number of other methods were proposed for fairly accurate simulation of speckles in OCT scans. Some of them used the Monte-Carlo approach to description of propagation of OCT-beam photons in a turbid scattering material [24].

Other approaches used the wave description of OCT signals, either in an approximate form [25] or using fairly accurate simulations of focused OCT beams utilizing either formalism of Gaussian beams represented in spatial domain [26] or a rather general angular-spectrum description [27]. In the context of testing attenuation imaging methods it can be stated that despite fairly accurate accounting for the interference nature of OCT speckles such models quite often did not consider the decay of OCT beams. Obviously, in the case of spatially uniform non-zero attenuation its influence could be modeled by applying a simple depth-dependent exponential factor to simulated OCT images. However, the assumption about spatially-uniform attenuation is oversimplified and does not look adequate for solving diagnostic problems using analysis of OCT scans in application to real biological tissues.

In what follows we describe a rather simple but efficient numerical approach to creation of fairly realistic OCT scans that can be used as “digital phantoms” for testing various methods of OCT-based optical attenuation imaging. The proposed approach readily allows one to account for main features of OCT scans. In particular, it correctly takes into account the interference nature of OCT speckles (i.e., the “speckle noise” intrinsic to OCT scans), as well as naturally describes the point-spread function shape and the influence of inter-pixel signal leakage without making additional assumptions. The proposed “digital phantom” allows one to introduce arbitrary spatial dependences of the OAC. It is generally accepted and confirmed by numerous studies that in OCT the signal attenuation is dominated by the optical wave scattering rather than absorption as was an essential assumption for the approach to OAC estimation proposed in Ref. [15] and subsequent related studies. Besides the “speckle noise”, other measurement noises can also be controllably introduced in the proposed “digital

phantom". Other main parameters of the simulated tissue (first of all, density and brightness of scatterers), as well as key parameters of the OCT setup (first of all, the illuminating beam spectrum), can also be very flexibly varied. In the following section we describe the proposed model and, using real OCT scans for comparison, demonstrate the possibility to simulate a number of characteristic situations of spatial distributions of OAC. Then the developed "digital phantom" is used to give some instructive examples demonstrating the usefulness of the proposed simulation method for development of spatially-resolved mapping of OAC.

2 Simulation of Basic OCT Scans

For simulating basic OCT scans we apply the approach described in Refs. [28, 29]. We briefly recall that such a model corresponds to the most widely used in OCT case of weakly focused beams, for which the variation in the illuminating beam radius is insignificant within the visualized depth. The illuminating-beam spectrum of a spectral-domain OCT setup is characterized by the spectral shape $S(k_n)$, where k_n are the discrete wavenumbers localized around the central wavenumber k_0 . For each lateral position of the beam it illuminates discrete scatterers with axial coordinates z_j and scattering amplitudes A_j . The illuminated scatterers emit backscattered waves, the complex amplitudes of which are summed at the reception. The phases of the received waves are determined by the phase angles acquired during the forth-and-back propagation. Summation of a discrete number of spectral components yields a pixilated 1D scan (A-scan) with the pixel amplitudes $A(q)$ given by the following expression [28]:

$$A(q) = \sum_j \sum_n S(k_n) A_j \exp(i2k_n z_j) \exp(-i \frac{2\pi n}{H} z_q). \quad (1)$$

In Eq. (1) the maximal unambiguously imaged depth H is determined by the distance δk between the neighboring spectral component in the source spectrum $S(k_n)$, such that $\delta k = k_{n+1} - k_n = \pi/H$. The axial positions z_j of the scatterers are random and continuous, whereas coordinates z_q of pixels in the pixilated A-scan are discrete and equidistantly located within the imaged depth H . It was demonstrated in Refs. [27, 29] that for weakly focused beams, one may take into account the amplitude shape of the beam cross section to describe how the scatterers enter and leave the illuminating beam when the latter is scanned laterally. The scattered-signal amplitude A_j takes into account the lateral position of the scattering particle within the illumination beam cross section. At the same time the neglect of phase variation within the beam cross section does not appreciably affect the simulated scans [27]. Such a method of OCT-scan simulation correctly takes into account the interference of waves backscattered by scattering particles located within every sample volume, the sizes of which are determined by the beam diameter and the coherence length that in turn is determined by the total spectral

width and form of the source spectrum $S(k_n)$. The interference nature of speckles in OCT in earlier models (e.g. [21]) was simulated via summation of a number of phasors with random phases. However, unlike simple summation of several phasors, the discussed here approach fairly rigorously considers interference of waves scattered by individual scatterers, so that speckle properties can be fairly correctly found for a chosen set of OCT-system parameters (illuminating-beam geometry and spectral properties of the optical source, density of scattering centers and statistics of their scattering amplitudes). The point-spread-function shape, including the effects of signal leakage among neighboring pixels are naturally obtained for both axial and lateral directions. In the lateral direction this is determined by the illuminating beam diameter and the degree of self-overlapping during the lateral scanning. The degree of independence of pixels in axial and lateral directions is important for spatial averaging procedures that will be used in the following sections.

Fig. 1 shows the results of OCT-scan modeling based on Eq. (1). The 2D scan (B-scan in Fig. 2(a)) with sizes 256×256 pixels is simulated for an OCT system with a central wavelength of $1 \mu\text{m}$ in a tissue with the refraction coefficient $n = 1.3$. This corresponds to the often used OCT sources with the wavelength of $1.3 \mu\text{m}$ in vacuum, the spectral width is 100 nm . The physical sizes of the simulated B-scan are $1000 \mu\text{m}$ axially and $2000 \mu\text{m}$ laterally. The lateral step between the neighboring A-scans is $8 \mu\text{m}$ and the half-width of the illuminating beam with a Gaussian profile is $10 \mu\text{m}$. The number of scatterers distributed over the scan area is $128 \cdot 10^3$, which imitates a typical density of biological cells in biological tissues. A single amplitude profile shown in Fig. 1(b) corresponds to the axial direction shown by the dashed line in Fig. 1(a). The histogram of the pixel amplitudes for the B-scan shown in Fig. 1(a) is presented in Fig. 1(c), where the dashed line shows the histogram approximation by a Rayleigh distribution expected for the developed speckle pattern [21]. If necessary, a desired level of measurement noise can readily be imitated in the simulated complex-valued B-scans by adding to every pixel random complex quantities, for example, with Gaussian distribution of real and imaginary parts.

An important issue that should be pointed out is that the so-simulated speckle patterns still do not imitate the influence of attenuation, as well as other factors affecting the OCT-signal amplitude in real B-scans. Among the latter factors the most important are the influence of the so-called roll-off effect intrinsic to spectral domain OCT devices and the illuminating beam focusing/divergence. The latter somewhat affects the signal amplitude even in OCT systems with fairly weakly focused illuminating beams. Although the origin of these effects and the corresponding approximating equations for the roll-off effect and focusing are well understood [10, 11, 30], for real OCT systems, the parameters entering these equations usually are not known.

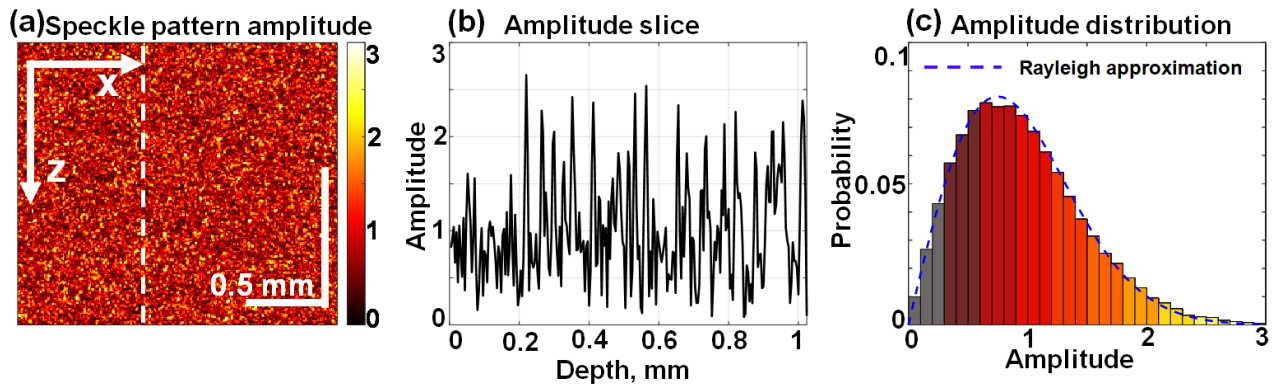


Fig. 1 Demonstration of speckle pattern, representative depth profile and speckle-amplitude statistics for the generated OCT scan. (a) Structural OCT scan shown in a linear scale. (b) Representative depth profile along the dashed line in panel (a). (c) Histogram of amplitude distribution.

In view of this, the corresponding correcting depth-dependent factors for a particular system can be determined experimentally. For example, the amplitude of the OCT-signal scattered from a plane boundary of a scattering phantom can be measured as a function of the boundary depth. Bearing in mind that the focus position depends on the optical wavelength dependent on the refraction index, one may place a layer of a transparent immersion liquid above the calibration sample. In such a case, one should verify that the illuminating-beam absorption in the immersion layer is either negligibly small or known from independent measurements. In what follows we assume that such preliminary calibration measurements are made and the depth-dependent correcting factor are applied to the OCT scan, so that in the absence of signal attenuation in the studied sample the OCT signal should have in average uniform amplitude like in the scan shown in Fig. 1(a). In the next sections we first consider how various depth-dependences of attenuation can be introduced in the formed “digital phantom” based on initially simulated B-scans with spatially uniform amplitude like in Fig. 1(a) and then the developed “digital phantom” is used to give some instructive examples demonstrating the usefulness of the proposed simulation method for development of spatially-resolved mapping of OAC.

3 Introducing Various Depth-Dependences of Attenuation in the Digital Phantom

There are two main significantly different reasons of optical signal decay for which numerous data can be found in literature. One reason is the optical signal absorption usually characterized by the absorption coefficient μ_a . The other reason is the signal decay caused by scattering and characterized by coefficient μ_s . For spatially uniform media, both attenuation mechanisms cause exponential signal decay described by factors $\exp(-\mu_a z)$ and $\exp(-\mu_s z)$, where z is the propagation distance. It is also assumed that other factors affecting the amplitude (like the above-mentioned influence of focusing or the roll-off effect) are absent or are duly compensated. In biological tissues the attenuation essentially depends on the optical wavelength. This fact

is taken into account when choosing the operating wavelength of OCT signals, so that the so-called transparency windows are chosen to increase the imaging depth. In particular, near infrared range is often chosen for creation OCT devices, e.g., one of often used wavelengths in OCT is 1300 nm. Numerous independent studies of optical signal attenuation in various media consistently indicate that in biological tissues the optical signal attenuation is strongly dominated by scattering rather than absorption, see, e.g., [13, 31, 32]. The statement about strong dominance of scattering over absorption is also related to OCT-signal attenuation. This fact is generally accepted and is used as an essential basic assumption for OAC-reconstruction methods widely used in recent years [15]. In terms of the above introduced attenuation coefficients μ_a and μ_s , the condition of strong dominance of scattering means that for most biological tissues one may consider that $\mu_s \gg \mu_a$, so that the energy loss during the forth-and-back propagation of the illuminating OCT beam is mostly caused by scattering.

Then following Ref. [15] to account for the attenuation process of the received OCT signal due to scattering, the following expression can be used:

$$I(z) = I_0 F(z) S(z) \theta(z) \cdot \mu_s(z) \cdot \exp[-2 \int_0^z \mu_s(z') dz'], \quad (2)$$

where $I(z)$ is the received intensity of the OCT signal; I_0 is the signal intensity incident on the biological tissue; $F(z)$ is the depth-dependent function describing focusing (confocal function); and $S(z)$ is the so-called roll-off function describing the depth-dependent sensitivity of the receiving array of detectors. Factor $\theta(z)$ is a coefficient characterizing the portion of total scattered radiation propagated back from scatterers and collected by the receiving array of OCT detectors. In some works, it is assumed that this portion should be constant in order to consider that the observed decay of the received OCT signal can be attributed to the scattering losses. However, it is clear that the influence of the unknown factor $\theta(z)$ is very similar to the effects of factors $F(z)$ and $S(z)$, so that the calibration measurement described in the previous

section can be used to determine and compensate the total effect of the product $F(z)S(z)\theta(z)$. Consequently, after such calibration and compensation the effective received signal acquires the simple form

$$I(z) = I_0 \cdot \mu_s(z) \cdot \exp[-2 \int_0^z \mu_s(z') dz'], \quad (3)$$

as if $F(z)S(z)\theta(z) = 1$. This equation still retains the depth-dependence of the received signal caused by the influence of the sought function $\mu_s(z)$.

Eq. (3) can be understood as the smoothed depth-dependence of each A-scan. The initial simulated 2D scan shown in Fig. 1 on the contrary is depth-independent corresponding to the absence (compensation) of all factors responsible for depth-dependence (including attenuation, sensitivity roll-off typical of spectral-domain OCT systems and both geometrical factors $F(z)$ and $\theta(z)$).

Consequently, for simulation of the influence of OAC dominated by signal scattering, one may take a depth-independent B-scan initially simulated as shown in Fig. 1. Initially this file is simulated in the complex-valued form (with both amplitude and phase), so that for obtaining the intensity, the complex-valued scan should be pixel-by-pixel multiplied by its complex-conjugated value. Then in the so-obtained intensity image, the intensity of each A-scan must be multiplied by the factor

given by Eq. (3) for a chosen desired distribution $\mu_s(z)$ of the optical attenuation coefficient. In such a way, however, one obtains only intensity images, whereas the described simulation procedure allows for obtaining complete complex-valued scans affected by the signal attenuation. To this end, one should again take the scan shown in Fig. 1 in the full complex-valued form and multiply it by the square root of $I(z)$ given by Eq. (3). Certainly, the intensity of the so-obtained files is exactly the same. However, the complex-valued scan opens additional possibilities, for example, for introducing arbitrary motions of scatterers to imitate a series of OCT scans affected by tissue motions, or for applying of coherent methods of OCT-scans processing, combining of OAC mapping with other OCT-modalities, e.g. elastography and angiography, etc.

Figs. 2 and 3 show examples of intensity distribution for the so-simulated OCT scans corresponding to two characteristic forms of $\mu_s(z)$. Fig. 2 shows the B-scan corresponding to a uniform distribution of OAC for $\mu_s(z) = 5 \text{ mm}^{-1}$ with the monotonically decaying signal intensity as is clearly seen from Figs. 2(a) and 2(b), in which the depth-dependence for the logarithm of intensity is shown. This dependence in average exhibits invariable slope determined by the exponential function in Eq. (3). Figs. 2(c) and 2(d) illustrate the same behavior in the linear scale.

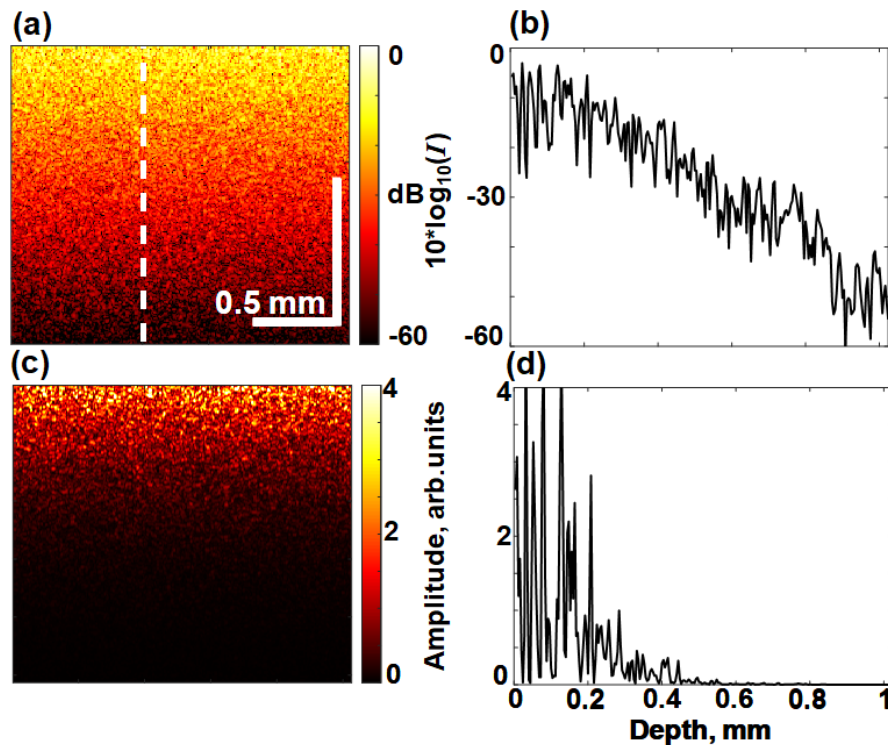


Fig. 2 Simulated OCT scans for spatially homogeneous OAC distribution with $\mu_s(z) = 5 \text{ mm}^{-1}$. (a) Structural OCT scan shown in a logarithmic scale. (b) Representative depth profile along the dashed line shown in panel (a). (c) The same scan as in panel (a) showing the OCT-signal amplitude in a linear scale. (d) The same depth profile as in panel (b) represented in a linear scale.

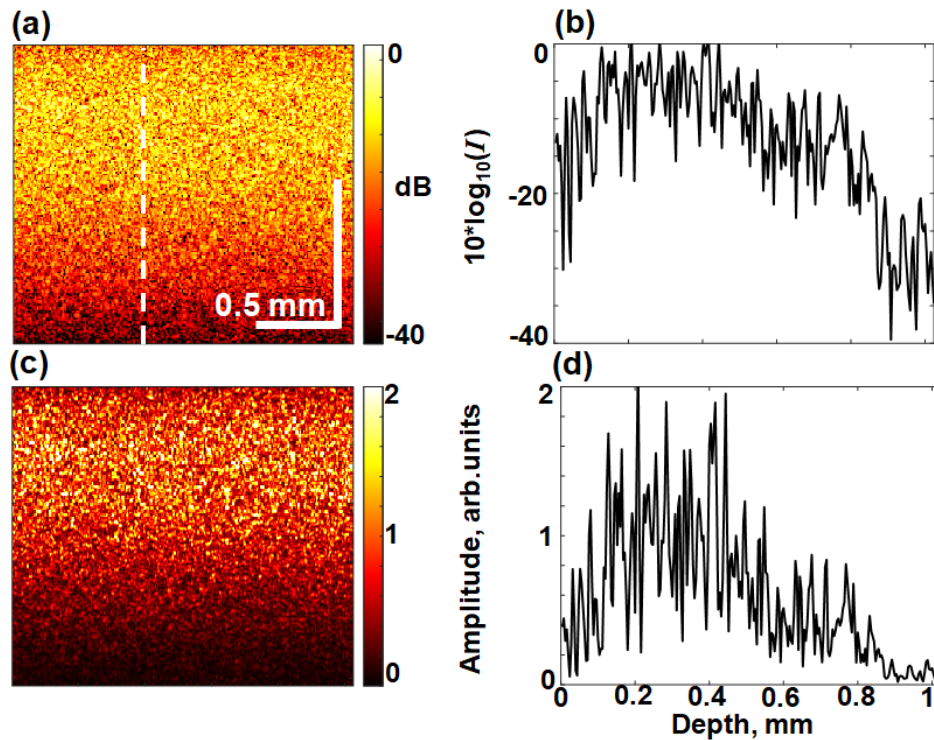


Fig. 3 Simulated OCT scans for spatially inhomogeneous OAC distribution with initial $\mu_s(0) = 0.25 \text{ mm}^{-1}$ and gradient $d\mu_s(z)/dz = 6.25 \text{ mm}^{-2}$ for which the received signal intensity demonstrates initial increase. (a) Structural OCT scan shown in a logarithmic scale ; (b) representative depth profile along the dashed line shown in panel (a); (c) the same scan as in panel (a) showing the OCT-signal amplitude in a linear scale; (d) the same depth profile as in panel (b) represented in a linear scale.

The next Fig. 3 demonstrates another characteristic form of $\mu_s(z)$. Namely, it is clear from Eq. (3) that the depth-dependence of the intensity is determined by the combined influence of the exponential factor and the pre-factor $\mu_s(z)$. It is clear that if this pre-factor sufficiently rapidly increases with the depth, such that

$$d\mu_s(z)/dz > 2\mu_s^2(z), \quad (4)$$

then intensity $I(z)$ may even increase with increasing depth. Fig. 3 illustrates such a situation and corresponds to coefficient $\mu_s(z)$ that linear growth as a function of z with the invariable rate $d\mu_s(z)/dz = 6.25 \text{ mm}^{-2}$ starting from $\mu_s(0) = 0.25 \text{ mm}^{-1}$. Figs. 3(a) and 3(b) are presented in logarithmic scale and show the intensity of the simulated B-scan and the depth dependence of intensity for a single A-scan. Figs. 3(c) and 3(d) show the same plots in the linear scale.

Note further that noise is always present in real OCT devices, and its presence introduces a bias in the received signal intensity, which should be adequately taken into account when estimating OAC [15, 16]. In the simulated complex-valued scans, a realistic measurement noise may readily be introduced by adding random, e.g., Gaussian quantities to the real and imaginary parts of the pixel amplitudes. Usually it makes sense to compare the mean noise intensity with the maximal intensity OCT B-scan usually occurred in the upper part of the scan. In

the following examples in Fig. 4, the so-defined signal-to-noise ratio amounts to $\sim 30 \text{ dB}$ in the upper part of the scans.

Notice further that by setting $\mu_s(z)$ to zero, it is possible to model an empty space above the studied sample, which is typical for non-contact imaging. Adjusting the simulated level and spatial distribution of attenuation, as well as the level of simulated noises one can imitate various types of real OCT scans of biological tissues. Some of such instructive real examples are presented in Fig. 4.

In Fig. 4 we use real OCT scans of homogeneous cartilage samples observed before and after 10 min of impregnation with an optical clearing agent (glycerol) like in works [33, 34]. For simulating scans, the attenuation and noise parameters were chosen to reproduce main features of the real scans. By applying a glycerol solution, the optical clearing effect was achieved in the upper layers of the cartilage sample, resulting in the formation of a gradient of the attenuation coefficient with the reduced scattering in the near surface layers and more intense scattering localized at an intermediate depth. This situation corresponds to sufficiently fast growth of $\mu_s(z)$ with increasing depth (as formulated in Eq. (4) and illustrated in the simulated Fig. 3) and is in contrast with the character of scattering before application of glycerol, when the brightest signal was localized near the sample surface.

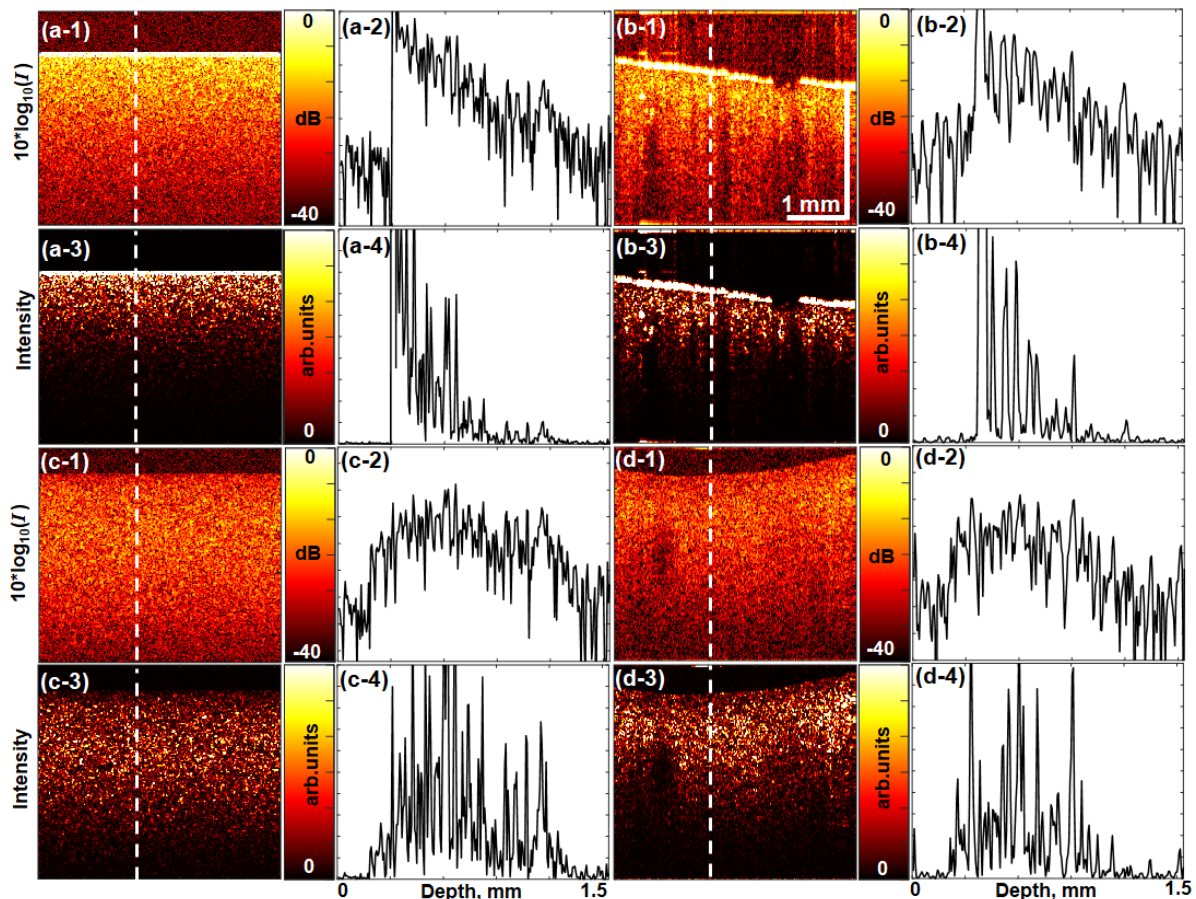


Fig. 4 Demonstration of similarity of the simulated (left column) and real (right column) OCT scans obtained for homogeneous cartilage samples before and after 10 min of impregnation with glycerol, respectively. Panel (a-1) is real OCT scan obtained before impregnation and (b-1) is the corresponding simulated scan shown in logarithmic scale. Panels (a-2) and (b-2) are the corresponding depth profiles along the dashed lines shown in panels (a-1) and (b-1). Panels (a-3), (a-4) and (b-3), (b-4) – the same plots as in panels (a-1), (b-1) and (a-2), (b-2) in the upper row but shown in the linear scale. Panels (c-1), (c-2) and (d-1), (d-2) are the same set of plots obtained after impregnation and the corresponding simulated plots shown in logarithmic scale. The bottom row shows a similar set of simulated and real post-impregnation plots shown in linear scale. Notice that initially the most intense signal occurred near the sample surface, whereas after impregnation with glycerol, the most intense scattering occurs at an intermediate depth.

4 Examples Illustrating Adequacy and Usefulness of the Described Model for Testing OAC-Estimation Methods

For OCT application in various biomedical problems it is of key importance to enable sufficiently reliable spatially resolved estimation of OCT-scan parameters. This requirement is common for various OCT modalities – OCT-based elastography [35–37], OCT-based angiography [38, 39], or parameters of speckle texture [8, 9, 40]. In this context, much attention in recent years has also been paid to the development of spatially-resolved methods for estimating the attenuation coefficient. A significant portion of such studies were stimulated by the appearance of paper [15] by Vermeer et al., where a formula for spatially-resolved estimation of OAC was proposed for usage in OCT. This formula is exact under certain conditions which are not always perfectly satisfied for real OCT scans. However, when these conditions are fairly well fulfilled,

method [15] of OAC reconstruction can be used as a reference one. By this reason for verification of adequacy of the described “digital phantom”, in the following section we demonstrate the results of OAC reconstruction by method [15] applied to the simulated OCT scans.

4.1 Correspondence between the Optical Attenuation Coefficient Adopted in the Simulated “Digital Phantoms” and Vermeer’s Reconstruction

In this section, compare the $\mu_s(z)$ profile adopted in the procedure of OCT-scan simulation using the procedure described in Section 3 with OAC reconstructed from the simulated scans using method [15] under nearly ideal conditions of the method operability. We emphasize once again that, when performing such a comparison, we assume that the geometrical factors and sensitivity roll-off entering Eq. (2) have already been compensated by

performing the calibration test as described at the end on Introduction. After such compensation the intensity profile can be affected only by the OCT-signal losses due to optical-signal scattering and eventual absorption in the tissue. Then the only remaining condition of the validity of approach [15] to reconstruction of OAC after the above-mentioned compensation procedures is that the OCT-signal attenuation is strongly dominated by scattering. This is precisely the case when the depth-dependence of the OCT-signal intensity reduces to Eq. (3).

Then in the absence of additive measurement noises and in the case of complete decay of the observed signal intensity $I(z)$ within the visualized region, for arbitrary form of $\mu_s(z)$, the following rigorously valid expression was derived in Ref. [15]:

$$\mu_s(z) = \frac{I(z)}{2 \int_z^\infty I(z') dz'}. \quad (5)$$

Physically Eq. (5) reflects the fact that the optical-beam energy passed through the depth coordinate z is equal to the energy loss caused by scattering during the optical-beam propagation from the current depth z to infinite depth.

It is important that Eq. (5) is written in the continuous form and is rigorously valid only at a semi-infinite interval. However, OCT scans are always acquired withing a finite depth interval $z \leq H$ and have a pixelated form. Thus, the discretized counterpart of Eq. (5) can be written as:

$$\mu_s(z_i) \approx \frac{I(z_i)}{2\Delta \sum_{j=i+1}^{j_H} I(z_j)}, \quad (6)$$

where index j_H corresponds to the maximal imaged depth H in the OCT scan and Δ is the axial inter-pixel distance (for brevity, in what follows called pixel size). Depth H is always finite, which leads to an unpleasant consequence intrinsic to Eq. (6). Namely, when $z \rightarrow H$ the estimate of $\mu_s(z)$ in Eq. (6) tends to a very big value $\mu_s(z) \rightarrow 1/(2\Delta)$ corresponding to a decay scale of the order of pixel size.

An important feature of both Eq. (5) for continuous representation and Eq. (6) written in the discretized form, is that the so-obtained spatially-resolved estimate of the attenuation coefficient $\mu_s(z)$ depends on the local values of $I(z)$. Therefore, when analyzing OCT scans characterized by the very strong variability of speckle intensities $I(z_i)$ like in the examples of speckle patterns shown in Figs. 3 and 4, function $\mu_s(z_i)$ reconstructed by Eq. (6) should also demonstrate strong variability. Consequently, more stable estimates of $\mu_s(z_i)$ can be obtained only using appropriately performed spatial averaging. The corresponding examples are shown in Fig. 5 using a simulated OCT image in which a spatially homogeneous distribution of $\mu_s(z)$ in the tissue was assumed as shown in Fig. 5(a-1). In the simulations shown in Fig. 5 for the moment we do not introduce additive measurement noises to show in the most clear form the influence of the “speckle noise”, i.e. the variability of speckle amplitudes intrinsic to OCT scans. Fig. 5(a-2) shows the corresponding simulated structural B-scan in which the pronounced “speckle noise” is visible. The corresponding very noisy map of OAC reconstructed using Eq. (6) without any averaging is shown in Fig. 5(b-1). Fig. 5(b-2) demonstrates a single vertical profile along the dashed line shown in Fig. 5(b-1).

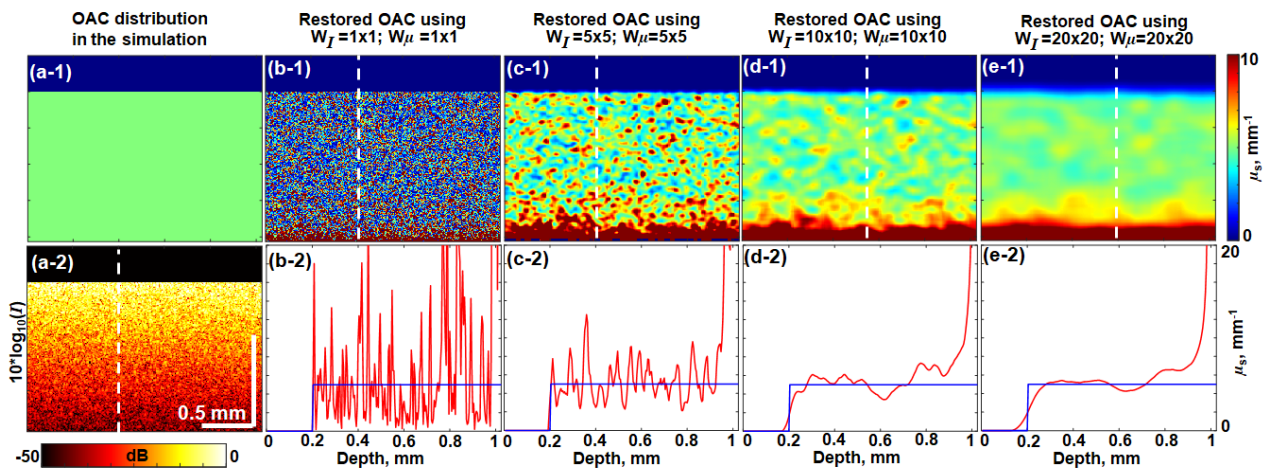


Fig. 5 Demonstration of OAC reconstruction using method [15] for several variants of preliminary averaging of intensity and subsequent averaging of reconstructed $\mu_s(z)$ using sliding windows 1×1 , 5×5 , 10×10 , and 20×20 pixels. (a-1) Distribution of OAC adopted in the model. (a-2) The corresponding simulated structural OCT scan. (b-1) Reconstructed OAC without averaging. Panels (c-2), (d-2), and (e-2) show reconstructed OAC using averaging windows with the sizes indicated above the plots, the white dashed lines indicate the positions of the depth profiles shown in panels (b-2)–(e-2). The OAC value inside the simulated tissue is $\mu_s(z_i) = 5 \text{ mm}^{-1}$.

The unaveraged profile of $\mu_s(z_i)$ reproduces the strong variability of intensity of individual speckles inherited from the structural OCT scan shown in Fig. 5(b-2), so that the adopted in the simulation depth-independent profile of $\mu_s(z_i)$ is almost completely masked by the speckle noise. To improve the quality of the obtained OAC maps one may use spatial averaging. In the examples shown in Fig. 5 the two-step averaging was used: (i) averaging over window W_l was first applied to the intensity of structural OCT-scan shown in Fig. 5(b-2); (ii) then averaging over window W_μ was applied to the attenuation coefficient $\mu_s(z)$ initially estimated using the previously averaged intensity for every pixel via Eq. (6). The results of such two-step averaging are shown for several sizes of averaging windows 5×5 , 10×10 , and 20×20 pixels. The same window sizes $W_l = W_\mu$ were used both in steps (i) and (ii).

The maps of $\mu_s(z)$ and representative profiles shown in columns (c), (d), and (e) in Fig. 5 demonstrate that with the appropriately performed averaging, the method based on Eq. (6) demonstrates a good correspondence of the OAC distribution adopted in the simulations and estimated attenuation coefficient over the most part of the scans not too close to the scan bottom. At the same time Fig. 5 also clearly demonstrates the above mentioned artefactual feature intrinsic to the discretized Eq. (6). Namely, closer to the bottom of the OCT scan, the estimated OAC demonstrates the artefactual growth that was mentioned in the discussion of Eq. (6). Nevertheless, for the main portion of the imaged region, the assumed in the simulation distribution of $\mu_s(z)$ and the results of its reconstruction from the simulated “digital phantom” using Eq. (6) proposed in Ref. [15] demonstrate a very good coincidence. Increase in the averaging-window size on the one hand eliminates noisy deviations of the reconstructed OAC profile from $\mu_s(z)$ adopted in the simulation, but on the other hand the averaging smoothens high gradients of $\mu_s(z)$ as is clear from Figs. 5(d-2) and 5(e-2).

This coincidence confirms that the simulated “digital phantom” is quite adequate. Among the distorting factors, which may introduce biases in the OAC estimate by Eq. (6), the most important is the influence of various measurement noises (unrelated to the speckle variability that is already captured in the examples shown in Fig. 5). These noises affect both the numerator and denominator in Eq. (6). Another important factor is the influence of incorrect estimation of the denominator in Eq. (6) because of incomplete decay of the OCT signal withing the visualized depth. The influence of these distorting factors on the OAC reconstruction was discussed in a number of studies (e.g., [16, 17, 41–43]) starting from work [15] itself. These distorting factors can readily be introduced in the simulated digital phantom so that the efficiency and accuracy of the corresponding modifications of method [15] and possibly alternative methods can be studied in detail in highly controllable conditions with the possibility of flexible variations of all parameters.

4.2. Demonstration of the Speckle Noise Role in Estimation of Essentially Inhomogeneous $\mu_s(z)$ Distribution

The above-considered examples were related to $\mu_s(z)$ reconstruction in the simplest case of spatially homogeneous distribution of OAC. However, it is ability of spatially-resolved reconstruction which is usually considered as the main advantage of Vermeer’s approach [15] and its modifications. Although the basic Eq. (5) is formally exact, it was already demonstrated in Fig. 5 that the strong random spatial variability of the speckle pattern intrinsic to OCT scans may very strongly mask the regular character of the $\mu_s(z)$ even in the simplest case when OAC is in average invariable within the visualized region.

Fig. 6 shows a more complex spatial distribution of $\mu_s(z)$ which is characterized by presence of several layers by analogy with the example discussed in Ref. [15]. Here, we still do not introduce additive measurement noise to demonstrate in the most clear form the degrading role of OCT speckles in the reconstruction of OAC, for which the discretized Eq. (6) is used. To compare with the case of absence of speckles, we first generate the structural OCT image and then normalize to unity amplitude or all pixels, so that the scan remains pixelated, but variation in speckle amplitudes become completely eliminated.

The layered OAC distribution adopted in the simulations is shown in Fig. 6(a-1). The step-wise profiles of $\mu_s(z)$ are also shown by the blue solid line in Figs. 6(b-2), 6(c-2), and 6(d-2). The corresponding structural OCT scan is shown in Fig. 6(a-2). In the right part of panel (a-2) the speckle pattern is retained. The left part of this panel shows the image obtained by applying the influence of attenuation described by Eq. (3) to the initial scan with normalized speckle amplitudes. The results of the OAC reconstruction via discretized Eq. (6) are shown as color coded maps in Figs. 6(b-1), 6(c-1), and 6(d-1). The left part in these maps corresponds to the left part of the structural image Fig. 6(a-2) with eliminated speckle-amplitude variability and the right parts are obtained with retaining of OCT speckles. The respective representative profiles of OAC reconstructed using Eq. (6) are shown in Figs. 6(b-2), 6(c-2), and 6(d-2) for three variants of spatial averaging similarly to Fig. 5. The profiles obtained with retaining of speckles are shown by solid red lines and the profiles found after elimination of the speckle variability are given by dashed black lines. It is clear that pixel-to-pixel reconstruction without averaging similarly to Fig. 5 yields OAC distributions strongly degraded by the speckle influence. The profiles of $\mu_s(z)$ obtained with normalization of speckle amplitudes are much closer to the profile adopted in the model with a clear step-wise structure even without averaging.

However, even for the OCT image with eliminated speckle noise, the finite depth used for reconstruction of $\mu_s(z)$ in the discrete Eq. (6) leads to appearance of deviation of the reconstructed OAC from the actual $\mu_s(z)$ distribution adopted in the simulations.

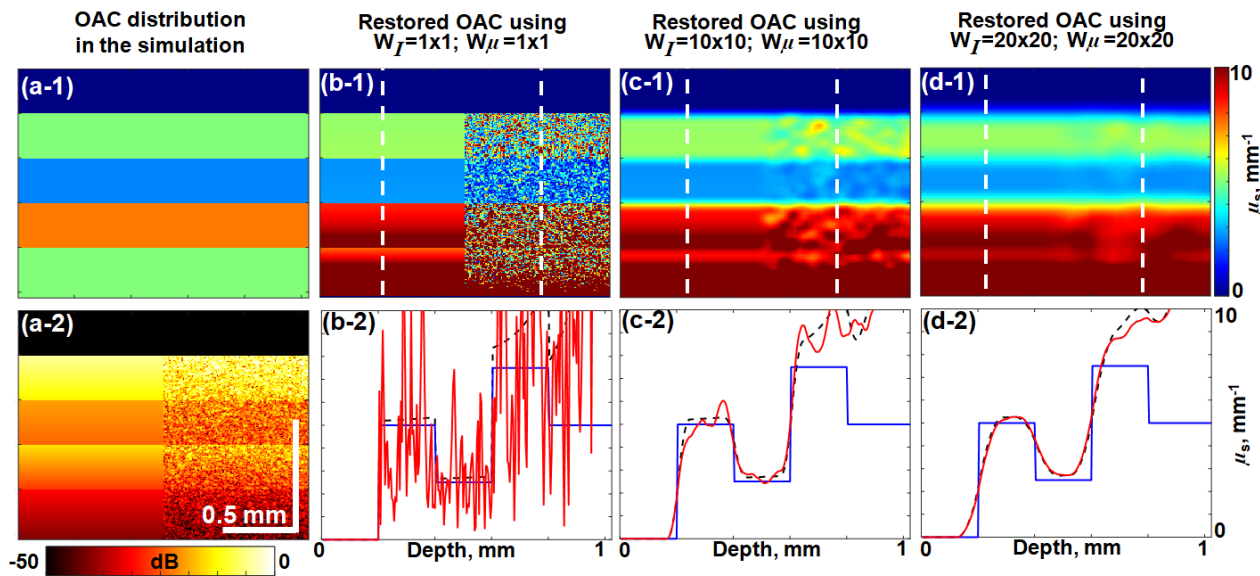


Fig. 6 Influence of the “speckle noise” on the reconstruction of spatially-inhomogeneous distribution $\mu_s(z)$ using approach [15] in the absence of additive measurement noises. Panels (a-1) and (a-2) show the OAC distribution adopted in the simulations and the simulated OCT scan, respectively. The OAC values in the five layer in panel (a-1) in the direction downward are 0, 5, 2.5, 7.5, and 5 mm^{-1} . The reconstructed 2D distributions of OAC are shown in panels (b-1), (c-1), and (d-1) without averaging, with averaging window 10×10 pixels and 20×20 pixels, respectively. The corresponding depth profiles of OAC for the positions marked by dashed lines in the 2D scans, are shown in panels (b-2), (c-2), and (d-2) by red lines. The blue lines in panels (b-2), (c-2), and (d-2) show the stepwise OAC profile adopted in the simulation.

This deviation is due to underestimation of the denominator in the discretized Eq. (6) in comparison with the exact continuous form of Eq. (5). Close to the image bottom this underestimation becomes especially strong causing rather strong deviations of the reconstructed and actual $\mu_s(z)$. Various modifications of Eq. (5) are discussed in the literature to eliminate this underestimation of the denominator (e.g. [16]). Such modified methods can also be tested using the “digital phantom” described here and the corresponding comparison of various modified approaches may be presented elsewhere. Returning to the present consideration, we emphasize once again that the speckle noise strongly degrades the quality of the spatially-resolved reconstruction of $\mu_s(z)$ as demonstrated in Fig. 6(b-2) and the right half of OAC map in Fig. 6(b-1). Averaging may help to significantly suppress this speckle-induced degradation, but this suppression is attained at the expense of reduction in the spatial resolution. The described “digital phantom” also makes it possible to quantitatively estimate the detectable contrast in the variable $\mu_s(z)$ for various sizes of averaging windows.

One may also mention that even if the reconstructed OAC distribution without averaging is rather noisy, the regions with significantly reduced OAC (more transparent regions in structural scans) are reconstructed with a better contrast. In practice, obtaining of maximal resolution in the reconstructed OAC maps is important when the spatially-resolved OAC reconstruction is used for detection of lymphatic vessels in OCT scans. It is known that these regions are characterised by reduced scattering [44, 45]. Besides fairly evident discrimination

of such regions by reduced amplitude of signal, the spatially-resolved mapping of OAC can also be used for imaging lymphatic vessels [43]. The proposed “digital phantom” can be used for testing and assessment of ultimate possibilities of this approach. For illustration, we present Fig. 7 in which the adopted distribution of OAC comprises local regions in which the scattering is reduced by various degrees. These regions imitate fragments of lymphatic-vessel cross sections (small circles) and elongated segments of such vessels that are more typical for *en face* images, but here they are presented in B-scans for illustration.

The reconstructed 2D maps of OAC demonstrate that fairly small regions, in which the scattering coefficient is reduced by a factor of 2 and greater, can be rather clearly singled out even in the presence of the speckle noise and even without averaging. The moderate averaging, for the window sizes $W_l = W_\mu = 5 \times 5$ pixels, improves the contrast between the background tissue and “lymphatic vessels” almost without the resolution loss. However, too large averaging window $W_l = W_\mu = 20 \times 20$ pixels on the contrary completely degrades the resolution of the visualization of the vessels. In these examples the addition measurement noise is not introduced and for OAC reconstruction we use Eq. (6). Certainly the additive noises can readily be introduced, the background OAC can easily be varied in the simulated OCT scans. Thus, the efficiency of visualization of regions with reduced OAC can be tested for various modifications of Eq. (6) discussed in the literature to account for the noise floor and the influence of incomplete signal decay, the degree of OAC contrast, etc.

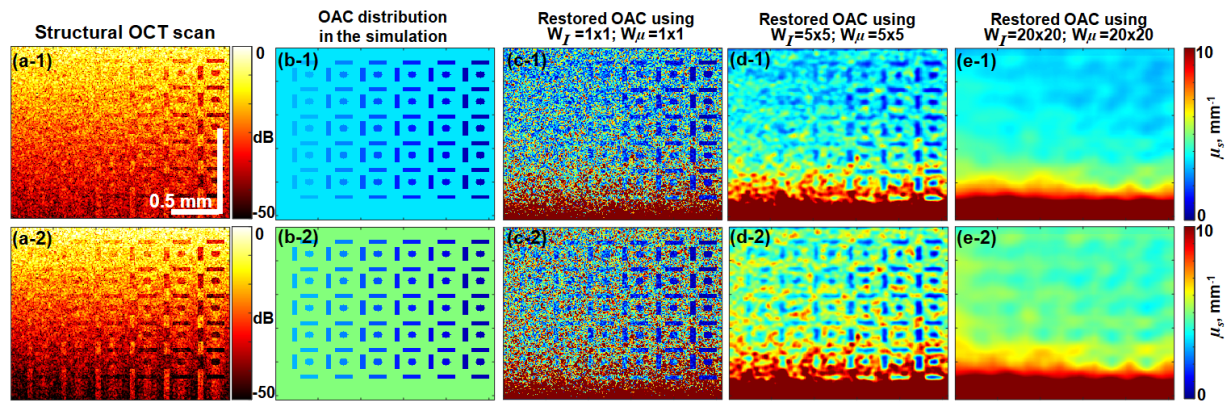


Fig. 7 Imitation of visualization of lymphatic vessels characterized by pronouncedly reduced scattering using spatially-resolved reconstruction of OAC. Column (a) shows the simulated structural scans, for which the adopted in simulation OAC distributions are shown in column (b). Columns (c), (d), and (e) show the OAC reconstruction based on Eq. (6) without averaging, for the averaging window $W_l = W_\mu = 5 \times 5$ pixels and $W_l = W_\mu = 20 \times 20$ pixels, respectively. The two rows correspond to the two different background values of the scattering coefficient of 3.5 mm^{-1} and 5 mm^{-1} , respectively. In the regions imitating the lymphatic vessels, for both rows the OAC value decreases from 3 down to 0.5 mm^{-1} in steps by 0.5 mm^{-1} .

Here, we limit ourselves to the above-presented examples illustrating the convenience of the developed digital phantom. Results of its application for detailed comparative tests of OAC-reconstruction methods will be presented elsewhere.

5 Discussion

In the previous sections we described a fairly simple although reasonably realistic model of OCT-image formation corresponding to widely used weakly focused Gaussian beams that enable approximately depth-independent lateral resolution within the visualized depth of 1–2 mm typical for OCT. In such beams, even the neglect of lateral curvature of the phase front is fairly reasonable as demonstrated in Ref. [27]. However, for more rigorous (although somewhat more computationally demanding) representation of weakly focused beams, the influence of the Gaussian-beam phase-front curvature can also be taken into account, for example, using the approach described in Ref. [26]. Method [26] is convenient for simulating two-dimensional B-scans in the form of adjacent individually generated A-scans. If simulations of 3D stacks of B-scans are required, approach [27] can be efficiently applied. Furthermore, it is noteworthy that approach [27] allows one to simulate arbitrary types of OCT beams, including Gaussian ones with arbitrary focusing, as well as Bessel beams or other beam types if necessary. This flexibility of approach [27] is due to utilization of the angular-spectrum approach which is very universal and applicable to description of various types of OCT beams.

The above-mentioned methods described in Refs. [26–29] similarly to the method used in this study generate OCT scans in which initially losses of the illuminating beam are neglected, so that accounting for scattering-induced attenuation can be made using the procedures described above in Section 3. In principle, besides the OCT-signal losses due to scattering, one may also introduce additional exponential factor describing

the optical-signal losses caused by absorption during the forth-and-back propagation. However, in such a case the elegant approach to spatially-resolved OAC imaging proposed in Ref. [15] would not be applicable because it is based on the assumption of strong dominance of scattering-related losses.

Certainly, the possibilities of approaches [26–29] to OCE-signal formation are not limited to testing methods of OAC imaging, but can readily be used for developing other OCT modalities. In particular, simulation methods presented in Refs. [26–29] have already been efficiently used for perfection of OCT-based elastography [46–48]. Similar modeling was used for testing various OCT-based approaches to angiographic imaging, see e.g. [27, 29, 49]. Regarding simulations of elastographic and angiographic processing, of key importance is that methods [26–29] readily enable generation of large series of OCT scans with arbitrary displacements of scatterers from one scan to another. Such displacements may be either regular corresponding to a desirable form of strain distribution or caused by regular flows, as well as may relate to random Brownian-like motions or arbitrary combinations of regular and random displacements (see, e.g., [29, 47, 49]). For alternative approaches to OCT-signal modeling, such generation of multiple OCT scans with accounting for interframe scatterer displacements is not always feasible or may require very high computational costs like for Monte-Carlo methods. However, the advantage of Monte-Carlo methods is that they may rather naturally account for effects of multiple scattering, consideration of which is problematic for methods [26–29]. In the present form these methods are based on consideration of ballistic single-scattered signal in the backward direction as is assumed by the very principle of OCT operation. In this regard, multiple scattering is a degrading effect in OCT. However, there also other important (in many cases even more important) degrading factors intrinsic to OCT scans, such as speckle

noise, as well as typical of many situations effects related to decorrelation of OCT scans due to translational or strain-induced displacement of scatterers. Such factors can readily be accounted for in the wave-based approaches [26–29]. Understanding of those multi-factor effects (both degrading and information-bearing ones) is very important for development of such OCT modalities as elastography or angiography, OAC imaging, speckle-contrast analysis of OCT scans, etc. Possibilities of purely analytical description of the respective effects are strongly limited by simplest situations. Consequently, utilization of the modeling method discussed in this study, as well as related methods [26–29] open very convenient in the context of developing not only OAC imaging possibilities, but also for studying a broad range of other OCT modalities for application in both educational and research problems.

6 Conclusions

In the previous sections we presented a simple but efficient method to simulate “digital phantom” which can be conveniently used for comparative studies of performance of various methods proposed for OAC estimation using OCT-scan analysis. The described approach makes it possible to realistically simulate both speckle noises and other additive noises affecting OCT images. Besides, unlike the simple summation of several phasors with random phases, the described “digital phantom” correctly accounts for the effects of signal leakage among neighboring pixels due to the final axial size of the point-spread-function determined by the optical-signal bandwiding, as well as the finite lateral size of speckles. The latter is determined by the illuminating beam diameter and the degree of self-overlapping during the lateral scanning. These factors are also realistically accounted in the so-formed “digital phantom” and determine the degree of statistical independence of

neighboring pixels. The degree of this independence is important for choosing adequate sizes of averaging window because the results of averaging procedures strongly depend on the amount of independent averaged elements. The simulated OCT scans realistically imitate speckle structure of OCT scans, which makes it possible to assess the degrading role of speckles in various situations. Besides correctly reproducing variability of intensity, phase properties of both the signal from scatterers and additive measurement noise can also be reproduced in the described model, so that both coherent and incoherent methods of OCT-scan processing can be comparatively tested using the presented simulation approach.

A very important advantage of simulations is extremely high flexibility allowing one to controllably vary all important parameters in the considered problems, including both properties of the simulated tissue and parameters of the OCT setup used to image this tissue. In physical experiments such flexible and precise control of experimental conditions is much more difficult and expensive and may be even unfeasible. Thus the proposed approach and above presented demonstrations of its usage can be used for further perfection of OAC estimation methods in optical coherence tomography.

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Disclosures

The authors declare that they have no conflict of interest.

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Investigation of the Influence of Thermally Induced Methemoglobin on the Human Skin Optical Reflection

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Abstract. The relationship between the reflection coefficient of human skin at a wavelength of 980 nm and its temperature during exposure to laser radiation of the same wavelength has been measured for the first time in an in vivo experiment. An interpretation of the experimentally obtained results is provided within the framework of a seven-layer optical computer model of human skin. Association of the observed alteration in the reflection coefficient at the wavelength of 980 nm to the conversion of skin blood hemoglobin into methemoglobin is demonstrated. The results of this research may find application in the development of novel laser systems with feedback mechanisms and technologies for treating dermatological conditions, including telangiectasias. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: diode laser; reflection; human skin; computer model; heating; temperature.

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1 Introduction

Laser systems with feedback capability enable real-time adjustment of laser exposure parameters on biological tissue, depending on the desired therapeutic outcome [1]. The biological tissue condition monitoring allows to adapt to non-standard conditions during the operation and optimization of laser intervention timely. The thermal-optical feedback is most prevalent, which measures the temperature in treated area by the radiance of the heated fiber tip [2, 3]. However, ability of such feedback is limited to register changes occurring within the depth of the biological tissue. This limitation becomes particularly crucial in non-contact interstitial laser procedures in dermatology, where the delivery of maximum energy into deeper layers of the skin is imperative without compromising the integrity of superficial layers. This non-contact procedure usually applicable for the treatment of telangiectasias through laser sclerotherapy.

During laser sclerotherapy, the laser radiation is absorbed by the pathological blood vessel, elevating vessel temperature to approximately +80 °C [4]. Consequently, the vessel walls adhere together, leading to irreversible damage [5]. Various types of lasers are

employed for laser sclerotherapy, including neodymium lasers with second harmonic generation, krypton, copper vapor, alexandrite, diode lasers (with wavelengths ranging from 800 nm to 1000 nm), neodymium, and others [5, 6].

Laser treatment of telangiectasias can be categorized into single- and dual-wavelength approaches [5, 7–10]. Single-wavelength laser therapy presents several drawbacks, such as hyper- and hypopigmentation, treatment efficacy variability based on skin type, discomfort during the procedure, and limitations concerning the depth and size of treatable vessels [5, 8]. Dual-wavelength laser treatment of telangiectasias involves the use of a combination of wavelengths at 585 nm and 1064 nm for example [7]. This method relies on altering the skin optical properties through the dermis preliminary heating with a laser pulse at a wavelength of 585 nm. The dual-wavelength approach is characterized by higher efficacy compared to the single-wavelength method, absence of hyper- and hypopigmentation, independence from the skin type for treatment effectiveness, as well as the mitigation of other side effects. Furthermore, larger blood vessels exhibit better responsiveness to dual-wavelength treatment [7].

However, in adopting such an approach, it is crucial to possess knowledge and capability in measuring changes in the skin optical characteristics resulting from concomitant laser-induced effects, such as its laser-induced heating, to optimize the sclerotherapy process with minimal side effects.

The alteration of the skin optical properties during laser-induced heating may be influenced by changes in the concentration of its major chromophores, such as water and hemoglobin, as heating leads to an increase in blood flow and intensifies water evaporation from its surface [11, 12]. It is also known that hemoglobin begins to transform into methemoglobin at a temperature of approximately +50 °C [13]. Theoretical analysis of the hemoglobin and methemoglobin absorption spectra, as well as the human skin absorption spectrum, reveals that with a complete hemoglobin replacement with methemoglobin in human skin, the most significant increase in absorption occurs at wavelengths of 629 nm (by 112.5%) and 1105 nm (by 96.2%), while the greatest increase in transmission occurs at wavelengths of 441 nm (by 3.7%) and 574 nm (by 24.4%) [14]. The increase in absorption arises from the fact that in the near-infrared region of the electromagnetic spectrum, methemoglobin exhibits a higher absorption coefficient than hemoglobin. The elevation of methemoglobin concentration in the blood of the skin leads to change light absorption, both at the previously mentioned wavelengths [15–17]. In this context, a dual-wavelength approach, different from that described above, to increase the efficiency of sclerosis of deep-lying telangiectasia we can proposed. It involves preheating the skin with an infrared laser pulse to achieve maximal transformation of hemoglobin into methemoglobin. This alteration of the skin optical properties is designed to increase its transparency in the visible spectrum. Subsequently, a visible laser pulse is used for effectively sclerosis deep-seated pathological vessels. It is evident that the efficacy of any dual-wavelength approach can be augmented through the monitoring of temperature and optical characteristics of the skin during the impact of the first wavelength. This involves determining the conditions under which the impact of the second wavelength will be optimal. A diode laser with a wavelength of 980 nm can be employed as a source of infrared laser emission, as its radiation adequately penetrates the skin (penetration depth ~1.6 mm at the e^{-1} level [18]). Notably, the human skin absorption at this wavelength significantly intensifies, by 50.4%, when replacing hemoglobin with methemoglobin [14].

Unfortunately, authentic *in vivo* data on the measured dynamics of optical properties of human skin under the influence of 980 nm wavelength radiation and the ensuing laser-induced heating are absent in the literature. Consequently, experimental acquisition of these data is a pertinent task for enhancing the efficacy of laser sclerotherapy for deep-seated telangiectasias.

It is worth noting that non-invasive and contactless *in vivo* measurement of the dynamics of optical properties of human skin is simplest realized during the

assessment of the reflected signal at the wavelength of the laser radiation stimulating skin heating, in this case, at the wavelength of 980 nm including because that this approach eliminates the need to inject an optical radiation receiver into the biotissue.

Thus, the primary objective of this study is to experimentally assess the influence of laser-induced heating on the human skin optical properties *in vivo*. This influence will be studied by measuring the dependency of the intensity of light reflected by human skin at the wavelength of 980 nm on the heating of the skin stimulated by laser radiation with the same wavelength. Furthermore, the obtained results will be interpreted, and through optical computer modeling, we aim to evaluate the influence of changes in the concentration of major chromophores in human skin on its reflection of radiation at wavelength of 980 nm.

2 Materials and Methods

To perform *in vivo* measurements of the intensity of light reflected by human skin at a wavelength of 980 nm, an experimental setup was created (see Fig. 1(a)). The setup included a continuous wave laser source ALPH-01-“DIOLAN” (NPP VOLO LLC, Russia), operating at a wavelength of 980 nm with an average power of up to 25 W. The laser radiation was directed into a quartz fiber and focused on the volunteer’s skin using a collimator. The setup also comprised an infrared video camera BCK-756-USB (EVS LLC, Russia), capturing variations in the intensity of reflected light, thermal imaging camera FLIR C2 (Teledyne FLIR LLC, USA) for monitoring the skin surface temperature, and a computer. The laser radiation spot diameter on the skin surface was 17 ± 1 mm.

Twelve volunteers participated in the experiment, where the same area of skin on the arm underwent laser exposure (see Fig. 1(b)). Written consent for the experiment was obtained from all volunteers.

The laser exposure was conducted continuously, with an average output power of 15 ± 0.3 W at the collimator, continuing until the volunteer reported pain, at which point the laser was switched off. The infrared video camera and thermal imaging camera recorded the video of the laser radiation spot at a wavelength of 980 nm and the skin surface temperature, respectively. Subsequently, the video data were analyzed on a computer, synchronized in time, and each frame of the thermal imaging camera video was correlated with a frame from the infrared camera video. The maximum temperature in the frame was determined for each n frame of the thermal imaging camera video. The total intensity of all pixels was computed for each n frame of the infrared camera video. The intensity of light reflected by human skin at a wavelength of 980 nm was estimated using the equation:

$$I_R = I_n / I_1, \quad (1)$$

where I_1 is the total intensity of all pixels for the $n = 1$ frame, corresponding to the onset of laser radiation.

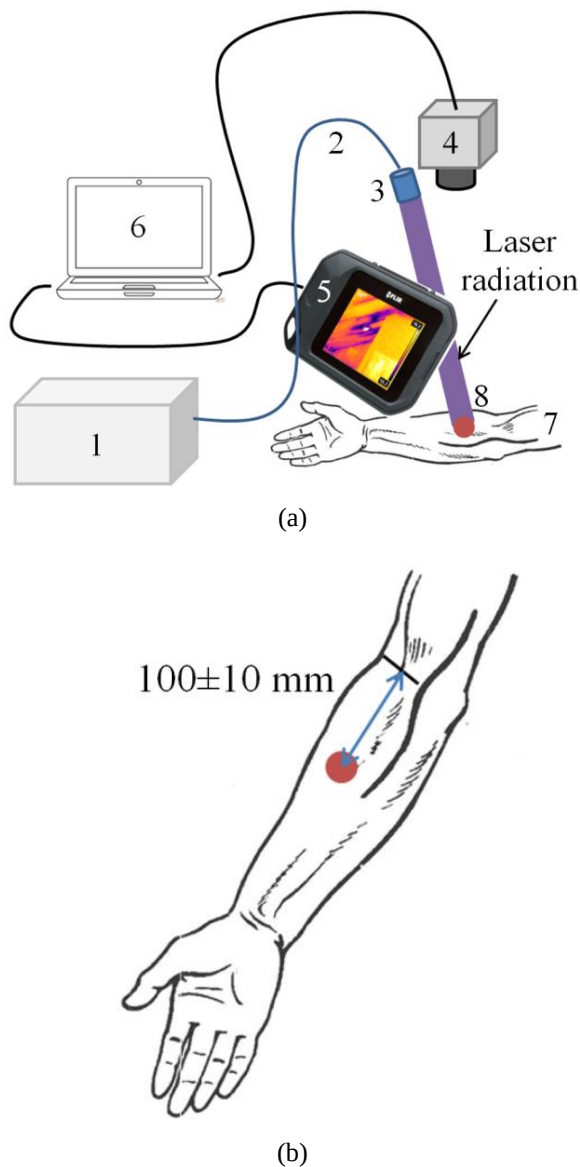


Fig. 1 Assessment of the impact of laser-induced heating on the optical properties of human skin *in vivo* by measuring the dependence of the skin reflection at a wavelength of 980 nm on the heating of the skin stimulated by laser radiation of the same wavelength: (a) experimental setup (1 – 980 nm diode laser, 2 – quartz fiber, 3 – collimator, 4 – infrared video camera, 5 – thermal imaging camera, 6 – computer, 7 – volunteer's arm, 8 – laser exposure site); (b) schematic diagram illustrating the location of the laser exposure site (red dot).

Through the analysis of correlated frames from the video recordings of the infrared camera and thermal imaging camera, dependencies of I_R on the maximum skin surface temperature at the laser exposure site were obtained for each volunteer.

Numerical model of the experimental setup (see Fig. 2) was created using the TracePro Expert ver. 7.0.1 software package (Lambda Research Corporation, USA) to interpret the experimentally obtained results. Subsequently, modelling was performed to determine the

simulated dependence of I_R at a wavelength of 980 nm on the concentration of methemoglobin in the blood of human skin, as well as on the concentrations of water and blood in the skin layers.

Seven-Layer Optical Computer Model of Human skin [10] was used during the simulation (see Fig. 3).

The methemoglobin concentration was set at 0%, 25%, 50%, 75%, and 100% relative to the total hemoglobin content in the skin layer in assessing the impact of methemoglobin concentration in the blood on the intensity of light reflected by human skin I_R at a wavelength of 980 nm.

The water concentration varied in the range of –20% to +20% relative to the initial value in evaluating the influence of water concentration in the entire skin on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. This corresponds to changes in water content in individual skin layers according to Table 1. Meanwhile, the concentrations of other chromophores were adjusted proportionally to the changes in water concentration.

The blood concentration was varied within the range of 0% to +100% relative to the initial value when assessing the impact of varying the concentration of blood in the entire skin on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. This corresponds to changes in the blood content in individual skin layers according to Table 2. Meanwhile, the concentrations of other chromophores were proportionally reduced with the increase in blood concentration. In the stratum corneum (SC) and living epidermis (LE) layers, the blood concentration remained at 0%.

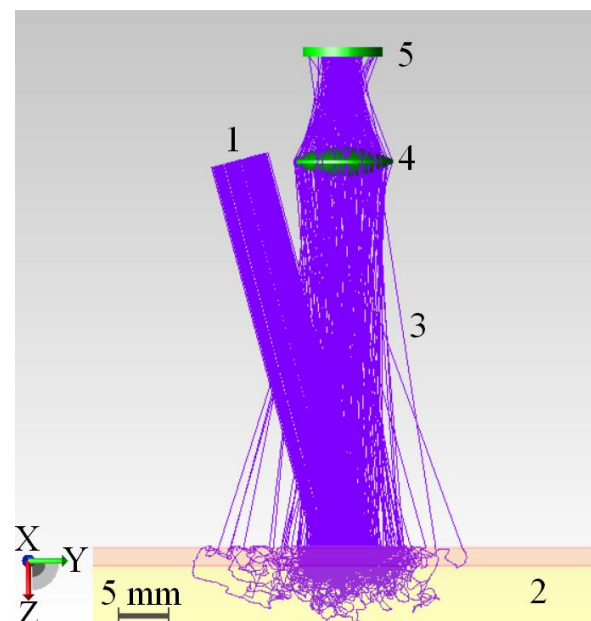


Fig. 2 Numerical model of the experimental setup (1 – a collimated beam of 980 nm laser radiation with diameter of 17 mm, 2 – a seven-layer optical computer model of human skin, 3 – light reflected by the skin with a wavelength of 980 nm, 4 – lens, 5 – receiving platform (100% absorber)).

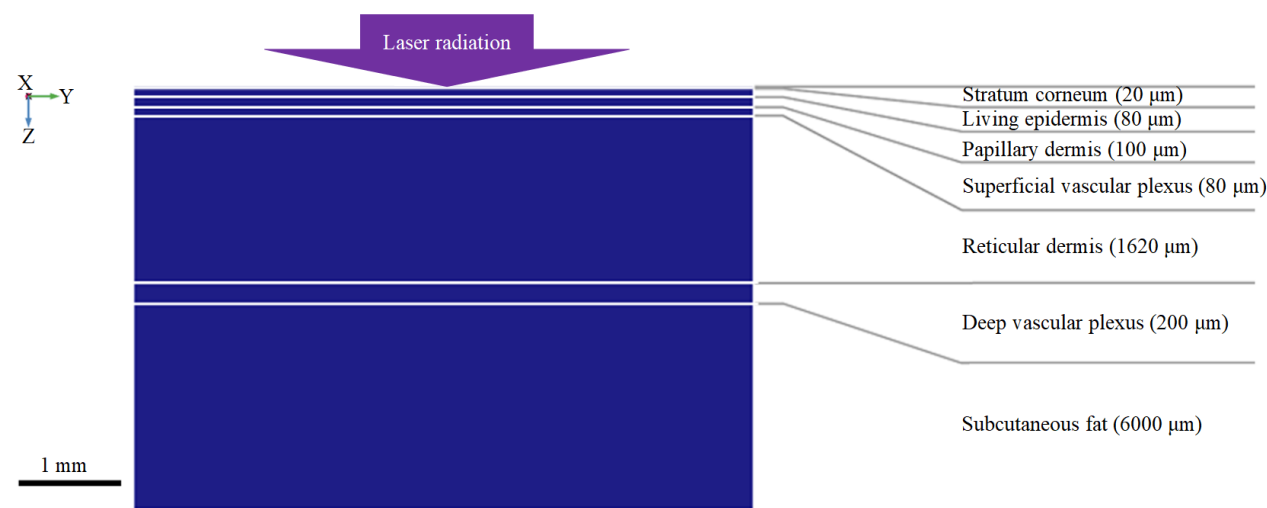


Fig. 3 Seven-layer optical computer model of human skin [10].

Table 1 Water concentrations in the layers of the seven-layer optical computer model of human skin used in the simulation.

Skin layer	Water concentration								
	−20%	−15%	−10%	−5%	0% (initial)	+5%	+10%	+15%	+20%
SC	4%	4.25%	4.5%	4.75%	5%	5.25%	5.5%	5.75%	6%
LE	16%	17%	18%	19%	20%	21%	22%	23%	24%
PD	40%	42.5%	45%	47.5%	50%	52.5%	55%	57.5%	60%
SVP	48%	51%	54%	57%	60%	63%	66%	69%	72%
RD	56%	59.5%	63%	66.5%	70%	73.5%	77%	80.5%	84%
DVP	56%	59.5%	63%	66.5%	70%	73.5%	77%	80.5%	84%

where SC – stratum corneum layer, LE – living epidermis layer, PD – papillary dermis layer, SVP – superficial vascular plexus layer, RD – reticular dermis layer, DVP – deep vascular plexus layer.

Table 2 Blood concentrations in the layers of the seven-layer optical computer model of human skin used in the simulation.

Skin layer	Blood concentration				
	+0% (initial)	+25%	+50%	+75%	+100%
PD	4%	5%	6%	7%	8%
SVP	30%	37.5%	45%	52.5%	60%
RD	4%	5%	6%	7%	8%
DVP	10%	12.5%	15%	17.5%	20%

where PD – papillary dermis layer, SVP – superficial vascular plexus layer, RD – reticular dermis layer, DVP – deep vascular plexus layer.

3 Results and Discussion

It was determined that the skin surface temperature at the laser exposure site reached $+48.8 \pm 0.6$ °C at the moment volunteers reported pain within the *in vivo* experiment.

Examples of frames from the infrared video camera and their corresponding frames from the thermal imaging camera, obtained during the *in vivo* experiment before and immediately after laser exposure (when pain occurred), are shown in Fig. 4.

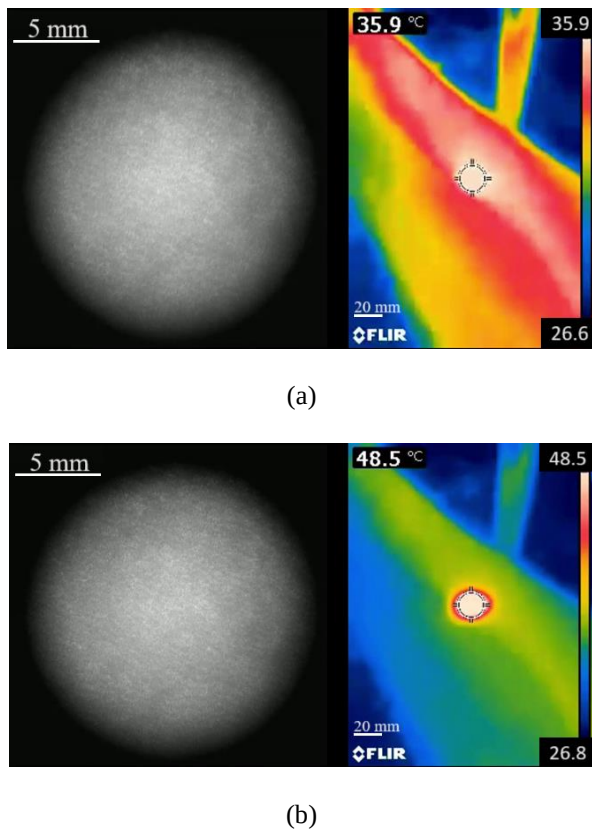


Fig. 4 Frames from the infrared video camera and their corresponding frames from the thermal imaging camera obtained during the *in vivo* experiment (a) before and (b) immediately after laser exposure (when pain occurred).

The dependency of the intensity of light reflected by human skin I_R at a wavelength of 980 nm on the skin surface temperature T_{skin} , derived from the analysis of the

sequence of frames from the infrared video camera and thermal imaging camera, is presented in Fig. 5.

From the dependences presented in Fig. 5 it is clear that with increasing temperature, the average intensity of light reflected by human skin I_R at a wavelength of 980 nm decreases with increasing skin surface temperature T_{skin} and by the end the decrease in I_R reaches $3.6 \pm 1.0\%$.

Optical computer simulation of skin reflection was performed to interpret the experimental results and assess the influence of skin chromophore concentrations on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. Simulated dependencies of I_R at a wavelength of 980 nm from methemoglobin concentration C_{MetHb} , changes in water concentration ΔC_{water} , and changes in blood concentration ΔC_{blood} in the skin layers was determined and represented on Fig. 6.

The dependences presented in Fig. 6 reveal that alterations in the concentration of major chromophores in the skin exert an influence on the intensity of light reflected by human skin I_R at the wavelength of 980 nm. Specifically, as the methemoglobin concentration in the skin increases, the intensity of reflected light I_R at 980 nm decreases (see Fig. 6(a)). This reduction is attributed to the fact that methemoglobin possesses a higher absorption coefficient than hemoglobin at the wavelength of 980 nm. Simultaneously, an elevation in the concentration of water within the skin leads to a decrease in the concentration of other light-absorbing components, resulting in an augmentation of the intensity of light reflected by human skin I_R at 980 nm (see Fig. 6(b)). Nevertheless, it is worth noting that variations in water concentration within the range of $\pm 20\%$ in the skin layers lead to a marginal alteration in the intensity of light reflected by human skin I_R at the wavelength of 980 nm, amounting to only $\pm 0.5\%$.

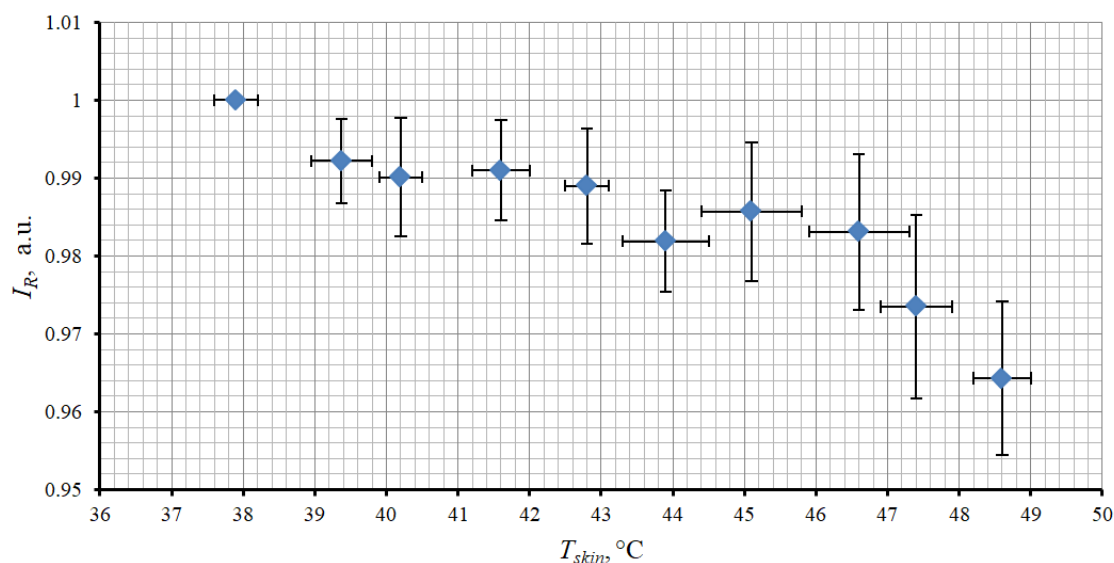


Fig. 5 The experimental dependence of the intensity of light reflected by human skin I_R at a wavelength of 980 nm (blue dots) with standard deviation (black bars) on the surface temperature of the volunteers' skin T_{skin} in the *in vivo* experiment.

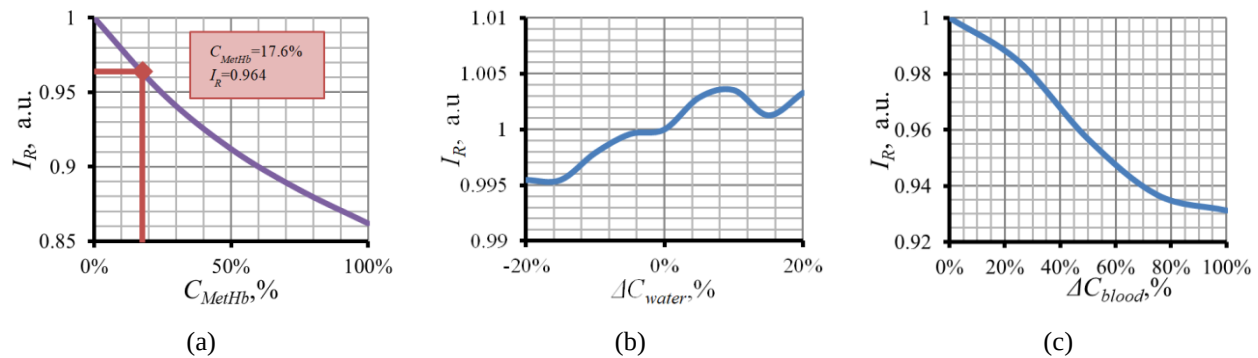


Fig. 6 Simulated dependencies of the intensity of light reflected by human skin I_R at a wavelength of 980 nm on the (a) methemoglobin concentration C_{MetHb} , (b) changes in water concentration ΔC_{water} , and (c) changes in blood concentration ΔC_{blood} in the skin layers.

Given this negligible change, when interpreting the results of *in vivo* experiments, the influence of water concentration fluctuations in skin can be disregarded. With an increase in blood concentration within the skin, the intensity of light reflected by human skin I_R at 980 nm decreases (see Fig. 6c). This reduction is attributed to the heightened presence of hemoglobin in the skin, which efficiently absorbs light at the wavelength of 980 nm. However, it is crucial to acknowledge that, in accordance with literature findings [7], an increase in blood flow in human skin occurs several minutes after the onset of heating. Therefore, when interpreting the results of this particular *in vivo* experiment, the impact of changes in blood concentration within the skin can be neglected.

Thus, by excluding the influence of changes in water and blood concentration within the layers of human skin, it can be inferred that the observed variation in the intensity of light reflected by human skin I_R at 980 nm, during the *in vivo* experiment is primarily determined by the formation of methemoglobin in the blood of the skin resulting from its laser-induced heating. Based on the simulated dependence of the intensity of light reflected by human skin I_R at 980 nm on the concentration of methemoglobin (see Fig. 6(a)) the change in the I_R is $3.6 \pm 1.0\%$ when the concentration of methemoglobin in the skin layers reaches a value equal to $17.6 \pm 4.9\%$.

4 Conclusion

It has been demonstrated that continuous diode laser radiation with a wavelength of 980 nm, heating the surface of human skin to a temperature of $+48.8 \pm 0.6$ °C,

leads to a reduction in the intensity of light reflected by human skin I_R at 980 nm by an average of $3.6 \pm 1.0\%$ for the first time in an *in vivo* experiment. Using the numerical model of the experimental setup and a seven-layer optical computer model of human skin, the influence of the concentration of the major chromophores (water, blood) in the layers of human skin on the intensity of light reflected by human skin I_R at 980 nm was investigated. Additionally, the impact of the transformation of hemoglobin into methemoglobin on the I_R was studied. It was found that changes in the concentration of water in the layers of human skin in the range of $\pm 20\%$ do not lead to a significant change in the intensity of light reflected by human skin I_R at 980 nm, doubling the blood volume (100% relative to the initial) results in a 7% reduction in the I_R , and complete replacement of hemoglobin with methemoglobin in the skin leads to a 14% reduction in the I_R . Considering experimental results and simulated dependence of the intensity of light reflected by human skin I_R at 980 nm on the concentration of methemoglobin in the skin obtained from optical computer simulation, at a skin surface temperature of $+48.8 \pm 0.4$ °C, the concentration of methemoglobin in the skin reaches $17.6 \pm 4.9\%$. This effect can be used to monitor changes in skin's condition during laser exposure, which will allow one optimizing the parameters of laser for improvement of treatment of skin in dermatology and cosmetology.

Disclosures

The authors declare that they have no conflict of interest.

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Enhancing Neurophysiological Analysis and Emotion Recognition with EEG Channel Spatial Metadata through Graph Neural Networks

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Abstract. The proposed architecture leverages the recording device's metadata by encoding the initial position of the recording electrodes into a graph structure which is then processed by the corresponding neural network architecture. This approach has proven its merit in neurophysiological applications such as P300 pattern detection and emotion recognition. Our experiments highlight the crucial role of the coordinate graph within the algorithm, which drastically influences the performance and efficacy of the model. Additionally, the versatility of our model is showcased through its consistent performance across diverse tasks, confirming its potential as a robust framework for future EEG-based research. Further analysis reveals that the incorporation of graph-based data alongside advanced optimization strategies markedly enhances the model's ability to generalize, making it a valuable asset for the neuroscience community. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: multimodal data integration; multivariate time series; electroencephalogram; spatial data; deep learning; graph neural network; P300 wave; emotion recognition.

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1 Introduction

Nowadays, analysis of multivariate time series is often reduced to the identification of so-called functional patterns [1], that is, features of time series behavior corresponding to some states of the system of interest to the researchers. Over the years, experts from various subject areas have already identified a number of functional patterns. The task of this paper is to prove the worth of utilizing the spatial information about the input device.

That information can be represented in the form of a graph containing data about the order relationship of the components of the multivariate time series, in our case it is the electrodes on the patient's head. Such an approach should improve the generalizing ability of the model under study. Using an architecture that incorporates separate temporal and spatial analysis could be useful for classifying electroencephalogram (EEG) data, as it allows the model to analyse both the spatial structure of

the brain activity and the temporal structure of the EEG data.

A common approach is to use a convolution neural network (CNN) architecture that incorporates separate temporal and spatial analysis, as it allows the model to analyze both the spatial structure of the brain activity and the temporal structure of the EEG data. The spatial convolutional layers could be used to extract features from the EEG data that reflect the activity of different electrodes, while the temporal convolutional layer could be used to extract features that reflect changes in brain activity over time. The fully-connected layer could then combine these features to produce a final classification decision. This approach with the current dataset was used in Ref. [2].

The main approach of CNN models was to apply a 1×1 convolution in order to get the weighted sum of the channels and work with the one-dimensional time series later. However, an alternative operation was proposed later. A graph convolution layer can be applied before the

weighted sum of the channels. Models with such processing blocks are called graph convolutional networks (GCNs). Their spatial processing block works with the graph data extracted from the input dataset. GCN applications to EEG data are still an active area of research, and there are a lot of modifications, which we will observe in this paper.

The GCN model could be trained on a dataset that includes both EEG data and measures of brain connectivity, such as functional connectivity (measures of the correlations between brain activity in different regions) or structural connectivity (measures of the physical connections between brain regions). The GCN could then be used to analyse this connectivity information and incorporate it into the model's prediction or classification decisions. Our approach is based on structural connectivity which could be extracted from spatial coordinates of electrodes. That information often accompanies the EEG datasets.

2 Related Work

The prevailing method for processing EEG data via graph convolutional networks is through a graph learning technique [3, 4]. In this method, the graph is constructed concurrently with the training process. Typically, the adjacency matrix is parameterized using the following Eq.:

$$A_{ij} = \frac{\exp(-\text{ReLU}(\omega^T |\psi_i - \psi_j|))}{\sum_{k=1}^N \exp(-\text{ReLU}(\omega^T |\psi_i - \psi_k|))},$$

where ψ_i and ψ_j represent the node features, N denotes the total number of nodes in the graph, and ω is a learnable weight vector. To optimize the learning of this weight vector, it is common practice to modify the model's loss function to ensure desirable properties such as sparsity and smoothness in the adjacency matrix:

$$L_{\text{gcn}} = \lambda \sum_{j=1}^N \|\psi_i - \psi_j\|_2^2 A_{i,j} + \|A\|_F^2,$$

where $\|\cdot\|_F$ signifies the Frobenius norm.

This technique bears similarities to the attention mechanism and is designed to highlight the most relevant channels in the time series data. However, unlike our proposed method, the graph constructed in this manner does not provide new information beyond what is obtained during the model's training phase. It essentially represents a sophisticated parameterization of the input data. Furthermore, the evolving and highly sparse nature of this graph has a negative impact on the model's interpretability.

The study in Ref. [5] introduced the Pyramidal Graph Convolutional Network (PGCN), which leverages prior knowledge regarding the brain's mesoscopic regions and electrode positions. In their work, the brain is segmented into either 7 or 2 mesoscopic regions [6, 7], and an

adjacency matrix based on spatial proximity is learned along with the other parameters of the model.

The spatial adjacency matrix is defined as:

$$A_{ij} = \begin{cases} 1 & \text{if } A_{ij} \geq 1, \\ \frac{\delta}{d_{ij}^2} & \text{if } 0.1 \leq A_{ij} \leq 1, \\ 0.1 & \text{if } A_{ij} \leq 0.1, \end{cases} \quad (1)$$

where d_{ij} represents the Euclidean distance between nodes i and j and δ is a hyperparameter referred to as the sparsity factor. Subsequently, the authors proposed converting this adjacency matrix into a learnable parameter without any other modifications of the method. Our experiments indicate that allowing gradient flow through the adjacency matrix selectively emphasizes the most informative channels and results in a modest enhancement of the model's performance.

Our goal is to validate the utilization of spatial-based graphs in EEG applications and highlight the interpretability and performance benefits of such an approach. Unlike the PGCN paper [5], we will concentrate exclusively on spatial features and explore potential refinements to this methodology.

3 Considered Dataset

3.1 BCI Competition III Dataset II

This section describes the dataset used in the work and the corresponding tasks, the solution of which, as neurophysiologists already know today, is associated with the functional pattern "wave P300".

There were 185 tests for each of the two subjects, A and B. The authors of the study have already proposed a partition of 85+100. Due to this partitioning, the random guessing method has only 2.8% accuracy on the test sample. We will use the partitioning provided by the authors to be able to compare our results with other algorithms applied on the same dataset. All EEG signals were collected using a 64-electrode scalp, filtered in the range from 0.1 to 60 Hz and digitized at a frequency of 240 Hz. You can read more about this in [1].

According to research by neurophysiologists, it is assumed, but not formally guaranteed, that highlighting the target symbol gives a "P300 wave". Although we know when to expect the P300 signal, its appearance depends on the subject, and the subject himself does not control this process either. The production of the "P300 wave" is not a phenomenon of consciousness, it occurs due to external stimuli (flickering of rows and columns). In addition, the "P300 wave" interferes with many other signals, correspondingly, its severity can vary greatly.

The problem is to determine whether the target character is contained in the highlighted row or column or not. In this case, the problem can be considered as a binary classification. The input is an EEG

corresponding to the highlighting of one row or column, and the output is a binary class label (whether the current highlighting is the target). As mentioned before, a 600 ms record is allocated for each highlighting, which is longer than the duration of the highlighting itself. The last object of the epoch will not be cut off, because each recording ends with a pause of 2.5 s, during which the matrix goes out.

3.2 Seed

For additional architecture validation we used the SEED dataset [8] for the emotion recognition task. That task has the same input data in the form of a raw EEG signal.

The SEED dataset is a publicly available EEG emotion dataset primarily designed for discrete emotion models. Signal data was downsampled to 200 Hz and band-pass filtered within the range of 0 to 75 Hz. The dataset encompasses three emotional types: positive, neutral, and negative.

For the EEG signals elicited by emotional stimuli from each subject within the dataset, the initial step involves capturing non-overlapping EEG signals of duration 1 second. Subsequently, we extract Differential Entropy (DE) features within five frequency bands (namely, δ (1–3 Hz), θ (4–7 Hz), α (8–13 Hz), β (14–30 Hz), and γ (31–50 Hz) rhythms) from each time slice and map them into a spatial matrix [9]. If we denote raw EEG data as X and suggest that the signals of each subband approximately follow a Gaussian distribution $X \sim N(\mu, \sigma^2)$, the formula could be written as follows:

$$\begin{aligned} DE(X) &= \\ &= \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \log \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) dx = \\ &= \frac{1}{2} \log(2\pi e \sigma^2), \end{aligned}$$

where π and e are constants, and σ^2 represents the variance of X .

4 Formula Problem Statement

P300 statement is a binary classification problem, where 1 means the presence of an impact, and 0 means its absence. One sample is a multivariate time series of fixed length, so each observation can be represented by a matrix of size $N \times T$, where N is the number of channels (electrodes), and T is the number of time intervals in which readings were recorded.

Analogically, *emotion recognition statement* is a classification problem with three classes. Negative, neutral and positive emotions stand for 1, 2, and 3 accordingly. In this setup authors also could not guarantee the appearance of a specific emotion from the subject. In that case the number of time intervals T represents the differential entropy for every frequency band δ , θ , α , β , and γ for every one-second slice of raw

EEG data. There is no neighbourhood relationship as well.

In the classic problem statement there is no neighbourhood relationship in the data. We can set some chain connection between the electrodes at best. This can be achieved with the channel sorting of the input matrix.

Having all the previously introduced terms at our disposal, we will rewrite the initial task as the graph neural networks statement. Let us denote the graph, which represent the initial neighborhood relationship, as G . G has a well-defined structure, and is also undirected, homogeneous, and static in time. Having a graph G with a one-dimensional time series associated with each vertex, we want to label it with one of two classes, where 1 represents the presence of an impact, and 0 represents its absence. That is, the problem is solved at the graph level with the teacher.

In our terminology, one sample is a graph. All objects have a structure G , but a unique one-dimensional time series is assigned to each vertex. Thus, as in the original problem statement, each observation is represented as a matrix of size $N \times T$, where N is the number of channels or electrodes, and T is the number of time intervals in which readings were recorded. However, this time a less trivial neighbourhood relationship G is set on the electrodes. Graph G is also fed to the algorithm. We will proceed to the construction of the graph G in the Section 6.

5 Proposed Architecture

As previously mentioned, we propose a model family that can be described with three types of modules: spacial processing block, temporal processing block and predictor block, which is a multilayer perceptron (MLP).

Our task is investigating of GCN capabilities, so the proposed architectures are quite simple. However, almost any existing EEG processing neural network could be expressed with such a structure.

The base model (Fig. 1) consists of 1×1 convolution as a spatial processing block and one-dimensional convolution as a temporal processing block. The predictor block is identical for all approaches and consists of multiplying the object vector by the matrix of the parameters being trained. Such an operation is defined only for vectors, but it will be applied at the last stage of object transformation, when both the graph in our problem and the matrix in the original one will be reduced to a one-dimensional embedding vector. Also, each convolution is accompanied by a nonlinear function ReLU [10].

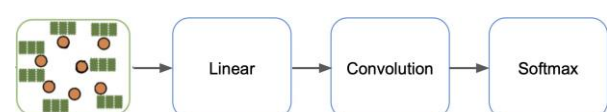


Fig. 1 Base CNN model.

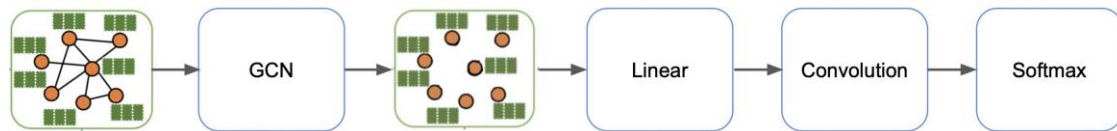


Fig. 2 GCN extended model.

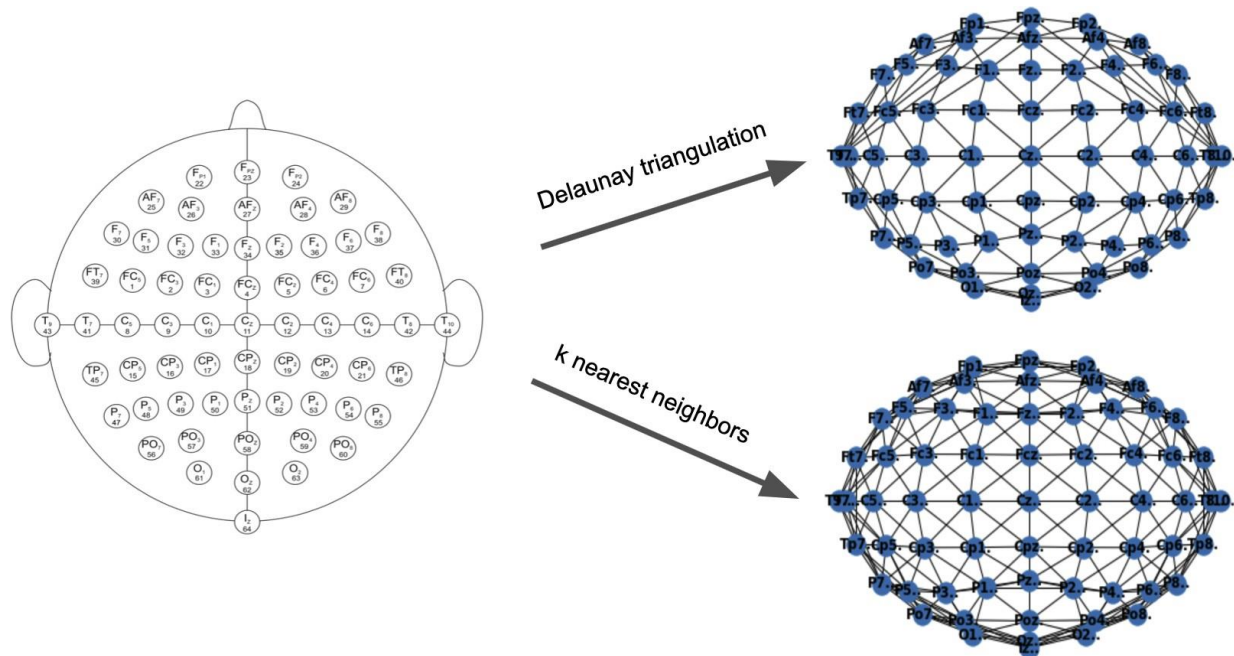


Fig. 3 10-10 mounting and obtained graphs.

Another approach is to add a graph convolution layer from GCN before the 1×1 convolution in the spatial processing block (Fig. 2). That could potentially improve the performance of the model by allowing it to capture more complex patterns of brain activity. The model can incorporate connectivity information into the input features. The GIN convolution from [11] was used because of its superior expressive ability.

6 Graph Construction

We will denote by G the graph representing the 10-10 standard. The vertices of this graph are electrodes, each vertex is encoded by a vector that contains the values of the potentials measured at each time. That is, each vertex of the graph is mapped to a time series recorded during the recognition of the P300.

In this paper, we obtain graph G in two ways: using Delaunay triangulation and the k nearest neighbours method, and compare the results (Fig. 3).

Medical data usually have fairly detailed specifications. For example, in our case, together with the data there was an article describing the procedure for collecting them, and a file with the coordinates of each electrode. It is the spatial information that we want to include in the neural network model in order to improve the quality of the received predictions.

Thus, by spatial coordinates we should get a graph G that characterizes the structure of the device used in

the experiments. We emphasize that the graph is built on a three-dimensional object, taking into account the form factor of the neurointerface, on a two-dimensional manifold.

We want to construct a graph with respect to the electrodes' neighbourhood, that is, there is an edge between two vertices if and only if they are neighbours in terms of coordinates. Now we will formalize the concept of a "neighbour".

Fortunately, humanity already knows how to solve such a problem, and we have considered two common solutions.

- The first approach is *Delaunay triangulation* [12]. This algorithm allows us to build a planar graph consisting only of triangles. Moreover, a circle can be described around each triangle, which will not include any other points of the triangulation, except for the vertices of this triangle. That is, the resulting graph will be sparse.

- The second approach is based on the classical *k nearest neighbours* algorithm, which calculates the distances to all objects in the dataset and searches for the k nearest among them. Since we are working with a real physical object, the Euclidean distance was chosen. The value of parameter k equal to 9 was selected experimentally.

Now, having a graph G , we can proceed to the formal formulation of the problem of pattern recognition.

7 Experiments

This section is devoted to the comparative analysis of selected approaches as applied to two datasets, which were detailed in Section 3. For the BCI Competition dataset, we adhere to the pre-defined split between training and testing sets. This standard enables us to benchmark our algorithms against existing strategies, including those developed in conjunction with the 2004 competition. The batch size is configured to 1024 samples. Adhering to the oddball paradigm, there are 2 positive class observations for every 12 objects, alongside 10 negative class observations, yielding a significant class imbalance of 1:6. As for the SEED dataset, it encompasses three balanced classes with a batch size set to 128, and the training partition is threefold larger than the testing partition.

We deploy consistent hyperparameters across both datasets: a learning rate of 10^{-4} and a weight decay of 10^{-2} . To bolster computational stability, we employed a learning rate scheduler with a *step* size of 5 and a γ parameter of 1. Both tasks converged within 500 epochs. For the “P300 wave” detection task we employed MSE loss, while for the SEED dataset CrossEntropy loss showed more promising results. With the training methodology established, we now embark on the empirical comparison of the proposed network architectures on the described datasets.

Initially, we evaluate our proposed models on the P300 dataset, aligning them with classical method of brain signal analysis such as gradient boosting.

For a point of reference, we utilize a baseline GCN model equipped with an identity matrix. Under this configuration, all models maintain an equivalent parameter count. However, the baseline model effectively translates to a graph void of connections. Additionally, our experiments incorporate Delaunay and KNN graphs. We also investigate a dense graph as described in Eq. (1), and a randomly initialized matrix drawn from a uniform distribution $\mathcal{U}(-\sqrt{k}, \sqrt{k})$, where $k = \frac{1}{N}$ and N represents the number of nodes. This is a standard practice for initializing the weights in the dense layers of neural networks.

For all architectures, excluding gradient boosting, we further employed gradient flow through the adjacency matrix to examine its effect as pointed out in Ref. [5].

Table 1 F1-score for binary classification problem.

Model	without gradient		with gradient	
	Subject A	Subject B	Subject A	Subject B
Gradient Boosting	28.03	24.04	—	—
Dense GCN	31.07	25.83	37.16	25.64
Baseline GCN	34.15	24.72	36.31	24.85
Delaunay GCN	34.93	25.25	37.20	25.26
KNN GCN	33.71	25.07	36.79	24.71
Random GCN	34.65	24.87	36.87	24.85

The F1-score was employed as the primary evaluation metric to accommodate the pronounced class imbalance observed within the first dataset. Insights drawn from Table 1 highlight that graph structure exerts a notable influence on model performance. While the KNN GCN showed relatively modest results, the Dense and Delaunay graphs consistently outperform the Baseline model across all experimental setups. As anticipated, gradient boosting yielded the lowest F1-scores.

Employing the gradient-based enhancement significantly improves results across models, so the distinction between their performance gradually reduces. Incremental improvements are notably observed in the baseline model endowed with an identity matrix. Notably, the random graph model delivers satisfactory performance across the board.

For Subject B, all models display modest results, with the Dense model exhibiting a comparatively advantageous edge. The narrow range of results may possibly be explained by poor dataset targets and model overfitting.

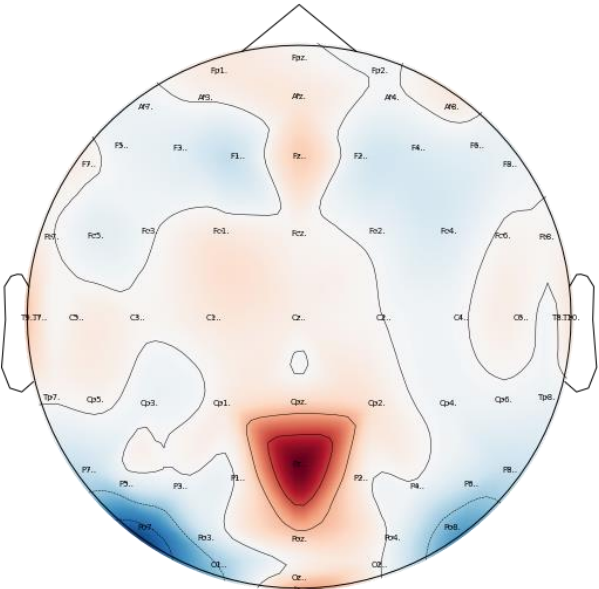


Table 2 Accuracy metric for emotion recognition problem.

Model	95% CI	
	without gradient	with gradient
Dense GCN	94.81 ± 3.74%	94.81 ± 3.74
Baseline GCN	83.83 ± 8.76%	90.37 ± 4.98%
Delaunay GCN	94.81 ± 3.74%	94.81 ± 3.74
KNN GCN	94.81 ± 3.74%	94.81 ± 3.74
Random GCN	87.41 ± 5.60%	94.81 ± 3.74
PGCN [5]	—	94.81 ± 3.74%

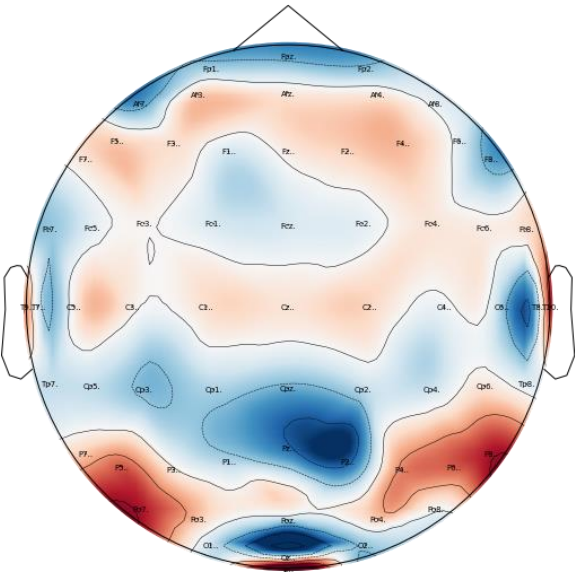


Fig. 5 The distribution of weights assigned to different features in the GNN model for the task of character recognition.

In addition to improving the quality of the model under consideration, the use of graph-based architecture allowed us to obtain more interpretable results. As stated in Ref. [13], the most informative electrodes in the recording device are Fz, Cz, P3, P4, Po7, Po8, Pz and Oz. In Fig. 4 we can notice that the basic architecture in our approach focused its attention on only three electrodes Pz, Po7, and Po8. While the graph neural network in Fig. 5 was able to recognize all of the above electrodes, except Fz and Cz.

The source of these visualizations is the 1 × 1 convolutional layer’s weight matrix, which yields higher absolute values to electrodes of greater importance during the weighted sum. In these figures, blue signifies negative values while red designates positive ones. However, our interest lies predominantly in their absolute values due to the inherent characteristics of the neural network training process.

The results in Table 2 were more unambiguous because the SEED dataset turned out to be too plain for

the considered model family. We used accuracy for multi-classification as the base metric for this task.

Distinct variations in model performance emerged with the baseline GCN, which lacked spatial information about channel structure, being an outlier. Noteworthy is the Random GCN, which achieved parity with the top-performing models with the gradient enhancement.

We further benchmarked our results against cutting-edge research in the domain of emotion recognition, notably [5]. The results show that our enhancements allow our models to rival state-of-the-art architectures such as PGCN in terms of accuracy, even though the high scores may suggest a saturation point for this particular dataset.

Inspection of the weight allocation maps further unraveled the models’ profound grasp of the data. There was a significant activation of nodes located in the temporal and occipital lobes and partial activation of nodes in the frontal lobe, aligning with the sources of different emotional impulses as stated in Ref. [8]. The spatial processing module in the GCN revealed activation of additional nodes, potentially contributing to the observed uptick in model accuracy.

8 Adjacency Gradient Interpretation

In this section, we will consider the adjacency gradient, which was derived through gradient flow from a random initialization. During the experiments, this was referred to as the “Random GCN”. To highlight the most substantial connections within the graph, we discretized the matrix values using the 95th percentile threshold.

As we can see from the Fig. 6, the matrix evolves over the course of learning. However, in the absence of explicit constraints, the matrix becomes asymmetric. Notably, the configuration that appears in the learned matrix is reminiscent of an attention mechanism, suggesting that the network identifies and prioritizes inter-channel connections that are important for the targeted task.

Dominating channels within the matrix, such as Fz, Po7, Po8, and Pz, coincide with four of the eight channels that neurophysiologists have pinpointed as crucial in the detection of the P300 wave [13], a finding that was also validated via analysis of the channel summation vector.

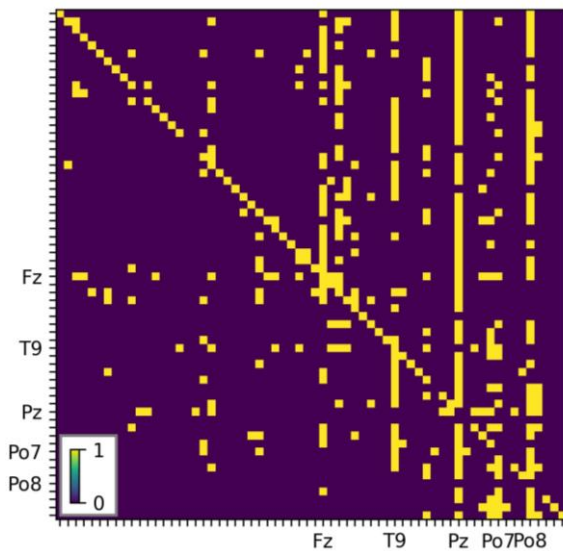


Fig. 6 Discretized version of the learned adjacency matrix highlighting connections between channels crucial for the task.

This congruence with neurophysiological understanding lends credence to the notion that the patterns discerned in the adjacency matrix are valid.

The autonomous functioning of this architecture segment as a distinct feature is promising. Future enhancements in parameterization and loss function adjustments may further refine the matrix structure, thereby augmenting the positive impact of this technique in the future research.

9 Conclusions

In conclusion, our study demonstrated the effectiveness of a unified model architecture in independent detecting the P300 wave and classifying emotions using EEG data. The incorporation of additional graph-based data and the utilization of specialized optimization techniques significantly enhanced the model's generalization abilities, as evidenced by the spatial filter weights and improved performance metrics.

Our experiments confirmed that graph structures play a crucial role in the success of Graph Convolutional Networks, markedly influencing model outcomes. The use of the gradient trick, in combination with the proposed approach, resulted in notable gains in model performance. This improvement allowed our models to compete with existing state-of-the-art models, suggesting a promising direction for future research in this domain.

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Disclosures

The authors declare no conflict of interest.

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Self-Assembled Porphyrin Nanoparticles Interaction Analysis with Albumin by Dynamic Light Scattering

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Abstract. We herein introduce the study of self-assembled porphyrazine nanoparticles photosensitizers in aqueous solutions with and without a polyvinylpyrrolidone carrier, respectively. For the first time, distributions of hydrodynamic radii for each of the samples separately were obtained, as well as corresponding changes in distributions for multicomponent systems in the presence of human serum albumin. For reliable interpretation of the obtained distributions, a statistical processing method adapted to biological samples was used. The study identified combinations of photosensitizer nanoparticles that could either efficiently interact with human transport proteins or, conversely, maintain their stability in their presence. Thus, the research conducted via dynamic light scattering revealed the potential for fundamentally different passive delivery methods of these nanoparticles: both with and without interaction with the main transport protein of human blood. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: dynamic light scattering; hydrodynamic radius; photodynamic therapy; self-assembled photosensitizers; porphyrazines; human serum albumin.

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1 Introduction

According to current data from the World Health Organization, about 9.3 million deaths from cancer were recorded in 2019. Malignant neoplasms are the second leading cause of death after cardiovascular diseases [1]. Cancer is one of the most important problems of the modern world. Among the treatment concepts for cancer, photodynamic therapy (PDT) can be highlighted – a modern, organ-preserving treatment method used in medicine. PDT is a local method for the treatment of various cancers, in which an intravenously administered photoactive drug, a photosensitizer (PS), accumulates in the tumor tissue in a higher concentration than in the surrounding tissues, and is activated after irradiation with light with a wavelength corresponding to its long-wave absorption peak. The photodynamic reaction that occurs in the presence of tissue oxygen causes the generation of singlet oxygen, which leads to tumor cells damage. The effect of reactive oxygen species is manifested by direct cytotoxic damage to malignant cells and destruction of blood vessels feeding the tumor. PS can also be used to diagnose cancer [2].

The process of delivering PS nanoparticles to tumor tissues is carried out by intravenous injection into the bloodstream, and currently two fundamental approaches can be distinguished that explain the effective passive targeting of nanoparticles:

1. Enhanced Permeability and Retention (EPR) effect which is still an active area of research and also a subject of debate [3, 4];

2. Methods based on the binding of human serum albumin (HSA), a transport protein, with PS particles [5–7].

In the first case, to realize the EPR effect, it is necessary to maintain the stability of the PS in the bloodstream, ideally obtaining nanoparticles of a deterministic size with a complete absence of interaction in the bloodstream. Under such conditions, the effect of targeted delivery to tumor tissues is expected to be maximized.

The second scenario represents a fundamentally diametrical concept in which, upon intravenous administration, the PS binds to blood proteins, leaving only a minor portion in a free state. Dye molecules attach to proteins due to electrostatic, hydrophobic, and

hydrogen interactions, as well as van der Waals forces. For instance, a single low-density lipoprotein molecule is capable of transporting up to 1000 molecules of a hydrophobic photosensitizer [8]. Tetraphenylporphines are mainly transported by albumin, while chlorin is bound approximately to the same extent to high-density lipoproteins and albumin [9].

The search for successful compounds that act as building blocks for PS is a subject of increased interest in the field of photodynamic therapy. The subjects of this study are water-soluble porphyrazine-based PS, capable of independently dissolving into stable nanosized micellar structures under the codenames PT_peg and S_27_Na. These compounds are heterocyclic analogs of magnesium phthalocyanine with reduced A3B-type symmetry [10]. S_27_Na nanoparticles contain a carboxyl group, which determines a number of important properties of the photosensitizer and is widely used in potential pharmacoforms [11, 12]. The polyethylene glycol group in the PT_peg structure additionally has the function of masking the micelle from its interaction with blood proteins, providing increased stability of nano-sized micellar structures in the bloodstream and greatly enhanced the drug-loading ratio of the single polymers [13, 14].

The optical method of Dynamic Light Scattering (DLS) enables the investigation of albumin molecule solutions and PS nanoparticles under conditions approximating the physiological. Using DLS, it is possible to study nanoparticles without altering their structure, making this method a practical standard when working with a such type particles [15–17]. In the study [5], DLS was used to analyze similar zinc phthalocyanine nanoparticles, demonstrating their complete disappearance in the presence of transport proteins due to disassembling of the PS complexes under modeled conditions. This work is the first to study a set of samples with a wide range of characteristics under passive load conditions, where polyvinylpyrrolidone (PVP) serves as a model drug. The separate binding of PVP to HSA was also demonstrated, which is a characteristic interaction between PVP and transport proteins [18, 19].

2 Experimental Method

To measure the nanoparticles sizes, as well as to monitor the dynamics of changes in the contributions of the molecules solution hydrodynamic radii, the dynamic light scattering method was used. The parameters of the PS dispersed in the liquid were measured using a Photocor Complex particle analyzer. The installation diagram is shown in Fig. 1.

The DLS method is based on the correlation function (CF) analysis of the number of photons over time recorded in a scattering volume in a given direction. The Brownian motion of scattered objects, in this case PS nanoparticles, causes temporary fluctuations in the intensity of scattered light. Thus, by analyzing the received signal, the correlator calculates an

autocorrelation function expressing the correlation of the scattered light intensity over time τ :

$$G(\tau) = \langle I(t)I(t + \tau) \rangle, \quad (1)$$

where averaging is performed over time t :

$$\langle I(t)I(t + \tau) \rangle = \lim_{\Delta t \rightarrow \infty} \frac{1}{\Delta t} \int_0^{\Delta t} I(t)I(t + \tau) dt, \quad (2)$$

where Δt is the accumulation time of correlation function. In this case, according with Onsager's hypothesis the relaxation of local concentration fluctuations is described by the diffusion Eq.:

$$\frac{\partial c(\mathbf{r}, t)}{\partial t} = -D \nabla^2 c(\mathbf{r}, t), \quad (3)$$

where $c(\mathbf{r}, t)$ – concentration, D – particle diffusion coefficient. Generally, DLS instruments export the normalized version of $G(\tau)$:

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2}. \quad (4)$$

The normalized second-order autocorrelation function $g^{(2)}(\tau)$ can be related to the normalized first-order correlation function $g^{(1)}(\tau)$ through the Siegert Eq. [20]:

$$g^2(\tau) = 1 + \beta [g^{(1)}(\tau)]^2, \quad (5)$$

where the first term of the sum is related to the baseline value (≈ 1) and the parameter β is the coherence factor that depends on the instrument and the scattering properties of macromolecules.

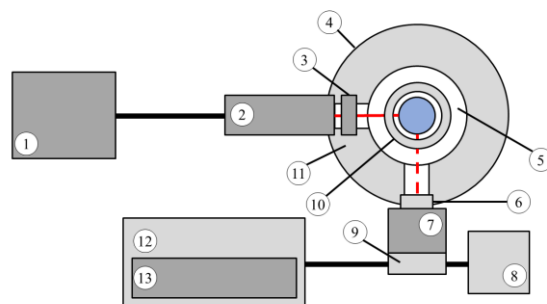


Fig. 1 Photocor Complex experimental setup schematic diagram: 1 – power supply, 2 – laser (445 nm or 647 nm), 3 – focusing lens, 4 – cuvette adapter, installed coaxially with the axis of the goniometer, 5 – goniometer console, 6 – receiving optical system (at 90°), 7 – photomultiplier operating in photon counting mode, 8 – special high-voltage power supply for PMT without parasitic correlations, 9 – amplifier-discriminator with a direct current bypass, 10 – thermostat, 11 – rigid base, 12 – personal computer, 13 – correlator directly connected to the personal computer.

The function $g^{(1)}(\tau)$ contains information about the movement of particles and for monodisperse particles the system decreases exponentially. In the general case (for polydisperse systems), $g^{(1)}(\tau)$ is an intensity-weighted integral over the distribution of decay rates $G(\Gamma)$:

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma) e^{(-\Gamma\tau)} d\Gamma, \quad (6)$$

where $G(\Gamma)$ is normalized: $\int_0^\infty G(\Gamma) d\Gamma = 1$, Γ the inverse correlation time:

$$\Gamma = \frac{1}{\tau_c} = D_t q^2, \quad (7)$$

where the wave vector of concentration fluctuations has the following form:

$$q = \frac{4\pi n}{\lambda} \sin \frac{\theta}{2}, \quad (8)$$

where n is the refractive index of the medium, λ is the wavelength of laser radiation, θ is the scattering angle. Using the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi\eta R_h}, \quad (9)$$

where k_B is the Boltzmann constant, T is the absolute temperature of the medium, η is the viscosity coefficient of the medium where particles of radius R_h are suspended. R_h – hydrodynamic or Stokes radius, calculated based on the spherical shape assumption of an object. Thus, based on the approximation of the scattered light intensity autocorrelation function using Eq. (7), it is possible to determine the particle diffusion coefficient and, ultimately, the sizes of the particles under study from the Eq. (9) [21–23].

Results are often processed using software provided by the supplier of the experimental setup, in particular, the DynaLS software program is recommended as the primary data analysis tool for DLS. However, in the case of working with a multicomponent system whose individual component contribution are unknown in advance, this approach can greatly increase subjective error. The need to select specific channel ranges, points of the correlation function for result processing, visual monitoring of residuals – forms of deviation from experimental data to the chosen model, and subjective regulation of the acceptable level of scattering intensity deviation from its average value can lead to significant distortions in the accurate ranges of the system's average hydrodynamic radius.

In order to avoid such errors, this work used a statistical method of data processing, a modified approach used in Raynals – software for analyzing DLS data using Tikhonov-Phillips regularization [24]. The main advantages of this approach are the uniformity of processing rules, as well as the working speed with large data. The final distribution was analyzed based on the results of averaging all measurements, which made it

possible to level out noise in the data and random deviations from the true size components contributions, simultaneously without the use of special corrective rules that could distort the true values.

3 Results and Discussions

Samples of PS nanoparticles (PT_peg and S_27_Na) were studied separately and using the synthetic polymer polyvinylpyrrolidone (PVP), which in this case acted as a passive load. In the future, therapeutic agents already known in medical practice, such as doxorubicin, could replace PVP as the payload. The interaction study of PS nanoparticles with the main transport protein of human blood HSA was conducted using 20% (200 g/L) albumin from “Octapharma” [25].

To resolve two peaks in the hydrodynamic radii distribution, their radius must differ by at least two times. Thus, the interaction between albumin and PS can be detected if a new stable and distinguishable peak appears with a size different from the peaks of HSA and PS.

During data processing, two main analysis approaches were identified. The empirical approach involves a sequential analysis of each measurement in the DynaLS program, based on the results of preliminary processing of all correlation functions obtained for the corresponding concentrations of the substance in an aqueous solution. In such cases, the dependence of the nanoparticles hydrodynamic radius reaches a plateau when concentrations are reached that are sufficient to exceed the contribution of the instrumental error – the phenomenon of an artifact peak [26]. To minimize subjective corrections, the following methods of averaging accumulated data were considered, taking into account the contributions of correlation functions individual components (Fig. 2):

1. distribution function for peaks of maximum contributions of averaged autocorrelation functions (Fig. 2a, b);
2. distribution function for all maxima of radius distributions (Fig. 2(c, d));
3. distribution function for all conducted measurements (Fig. 2(e)).

The most reliable and closest to the empirical approach was found to be the third method of averaging (Fig. 2(e)), which was chosen as the main approach for analyzing scattering data. To calculate the averaged radius distribution function, between 20 to 30 separate distribution functions were averaged. Similarly, the results about the sizes of the sample with PVP, as well as the sizes of nanoparticles S_27_Na and S_27_Na_PVP, were obtained (Fig. 3). Fig. 3 shows the corresponding graphs depicting the dependence of scattered light intensity on concentration during the measurements, which should have a linear dependence in accordance with Rayleigh's law:

$$I \sim n, \quad (10)$$

where I – scattered light intensity, n – number of particles per unit volume.

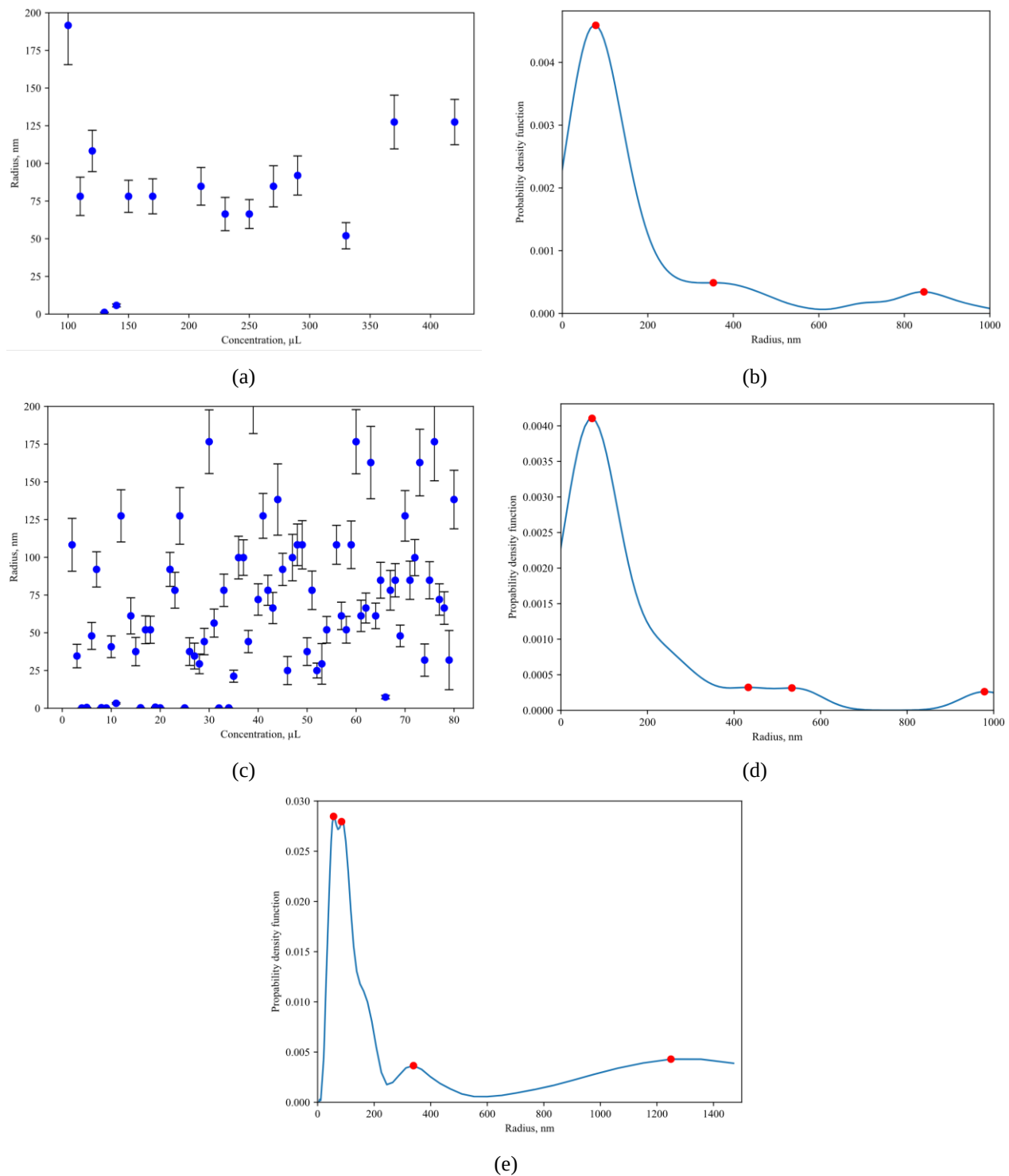


Fig. 2 Comparative analysis of averaging techniques: (a) all peaks of the radius distribution against the concentration of PS PT_peg. (b) Probability density function for the corresponding size of PS PT_peg across all peaks. (c) All peaks of the radius distribution by experiment number for PS PT_peg. (d) Probability density function for the corresponding size of PS PT_peg for averaged CFs. (e) Averaged probability density function for the corresponding size of PS PT_peg across all measurements. The red dots are the extremum points of the distribution density function.

The result nanoparticles sizes are given in Table 1.

It should also be noted that not all identified peak values are interpreted as the complexes sizes of the corresponding photosensitizer. For example, the last peak with order values of 1 μm and higher is an artifact peak that is not a real value and arises as a result of working

with highly diluted solutions. Fig. 3(d) shows a peak of several nanometers, representing free PVP particles.

Similarly, the HSA sizes components were measured. Fig. 4 shows the mean hydrodynamic radius distribution of HSA in aqueous solution as a function of concentration, as well as the intensity of scattered light.

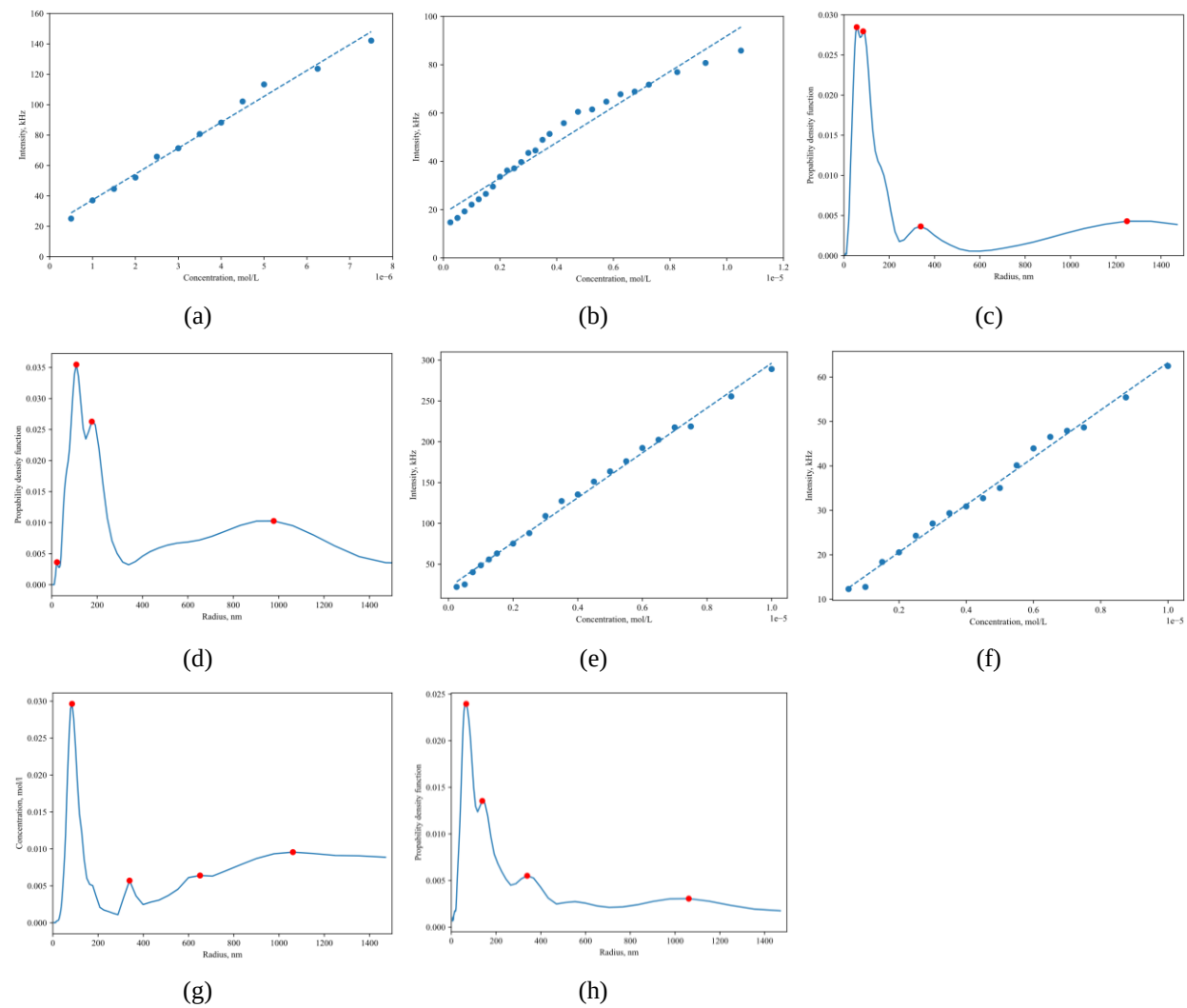


Fig. 3 Hydrodynamic radius determination for all samples: (a) Intensity vs. concentration of PT_peg. (b) Intensity vs. concentration of PT_peg_PVP. (c) Probability densities for the corresponding size of PS PT_peg. (d) Probability densities for the corresponding size of PS PT_peg_PVP. (e) Intensity vs. concentration of S_27_Na. (f) Intensity vs. concentration of S_27_Na_PVP. (g) Probability densities for the corresponding size of PS S_27_Na. (h) Probability densities for the corresponding size of PS S_27_Na_PVP. The red dots are the extremum points of the distribution density function. Blue dots – scattered light intensity for relevant concentrations. Dotted line – linear regression.

Table 1 Hydrodynamic radii values of PS nanoparticles.

Sample	Hydrodynamic radius, nm
PT_peg	45 ± 14
	112 ± 37
PT_peg_PVP	90 ± 28
	197 ± 40
S_27_Na	92 ± 38
	336 ± 24
S_27_Na_PVP	62 ± 23
	162 ± 36
	339 ± 52

Thus, the sizes of HSA in aqueous solution at pH ~ 7.5 are 3.6 ± 0.9 nm and 36 ± 10 nm respectively, where the first size corresponds to the literature data, and the second represents an energetically favorable aggregate of protein molecules.

Thus, having reliably determined the individual sizes contributions of nanoparticles and HSA separately, it is possible to draw conclusions about the presence or absence of a characteristic interaction through a similar analysis of HSA with the corresponding photosensitizer in an aqueous solution, having previously selected the concentrations so that the intensity contribution of the components is comparable and does not overlap the contributions individual particles. Table 2 presents the concentration ranges of the samples that were used in this study.

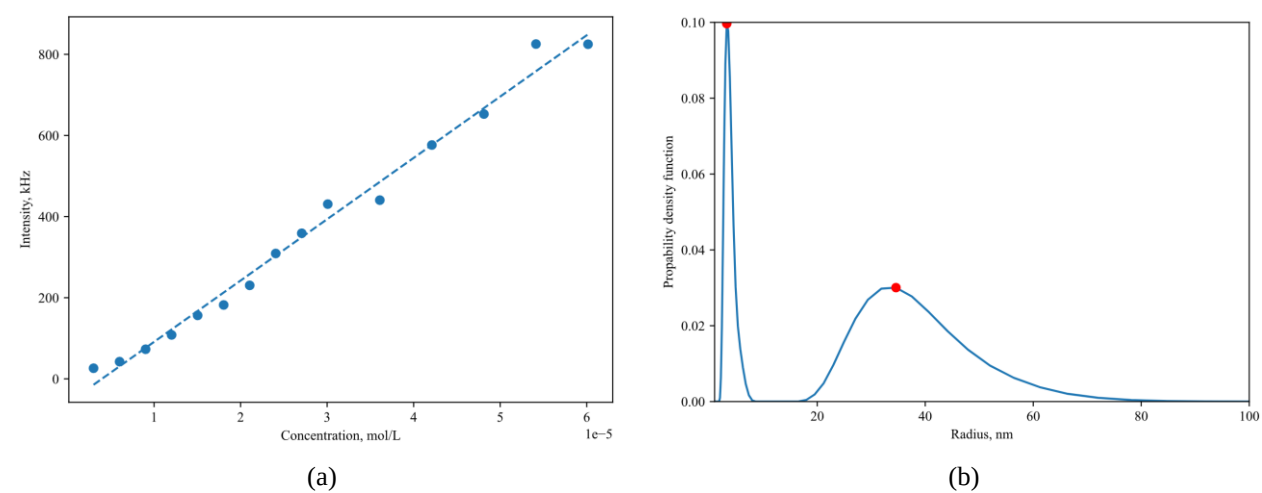


Fig. 4 Hydrodynamic radius determination for HSA: (a) Intensity vs. concentration of HSA. (b) Probability densities for corresponding size of HSA. The red dots are the extremum points of the distribution density function. Blue dots – scattered light intensity for relevant concentrations. Dotted line – linear regression.

Table 2 Ranges of samples limit concentrations.

Sample	Minimum concentration, mol/l	Maximum concentration, mol/l	Ranges of sample volumes, μ L
PT_peg	$1.25 \cdot 10^{-6}$	$1.5 \cdot 10^{-5}$	50–600
PT_peg_PVP	$1.25 \cdot 10^{-6}$	$1.5 \cdot 10^{-5}$	50–600
S_27_Na	$2.5 \cdot 10^{-6}$	$3 \cdot 10^{-5}$	50–600
S_27_Na_PVP	$2.5 \cdot 10^{-6}$	$3 \cdot 10^{-5}$	50–600
HSA	$1 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	14–40

Let us consider the probability density distribution functions for the hydrodynamic radii in the aqueous solution of PT_peg with HSA and PT_peg_PVP with HSA, respectively, presented in Fig. 5 (a, b). With a fixed concentration of HSA ($n = 3 \times 10^{-5}$ mol/L), the concentration of PS nanoparticles was increased, eventually reaching a PS/HSA ratio of 1:2. Fig. 5(c, d) highlights the size distribution functions of the nanoparticles separately for pure HSA, PS, and their solution at the maximum concentration ratio. Fig. 5(c) shows a repetition of the HSA size distribution peaks in HSA and PT_peg solution, with a slight increase in size. This increase is associated with a decrease in the influence of the artifact peak due to an increase in the total concentration of the solution. The absence of other peaks is due to the overlap of the signal from the sizes of nanoparticles; however, additional sizes are not observed, which indicates the absence protein interaction with the PT_peg sample. Fig. 5(d) shows a similar picture of the size distribution, but in this case a new peak appears that does not correspond to the sizes of either the protein or the PS nanoparticles. However, this size is not the result of the nanoparticles interaction with HSA. In this case, a corresponding peak in the interaction of

PT_peg with the HSA would be observed, instead we detect the formation of protein associates with free PVP particles. A similar distribution is shown for S_27_Na and HSA in Fig. 6, however, in this case, in the absence of PVP as a passive PS load, we see a new peak that does not correspond to either the individual sizes of HSA or S_27_Na, which indicates the interaction of nanoparticles with the main transport protein. In the case of S_27_Na_PVP, such a distribution is not informative, since it is not possible to identify specific peaks. Taking into account the interaction of S_27_Na NPs separately, as well as free PVP with HSA, a picture of multiple intensity contribution peaks was obtained over a large number of sizes, which cannot be resolved individually (Fig. 7(b)). For comparison, Fig. 7(a) shows the corresponding distribution of peaks in the case of individual S_27_Na PVP nanoparticles, where, in the absence of any interactions, repeatable and reproducible peaks are observed with increasing concentration of the substance. The rightmost peak is an artifact peak, which in this case does not represent the real size, but is typical when working with highly dilute solutions [26].

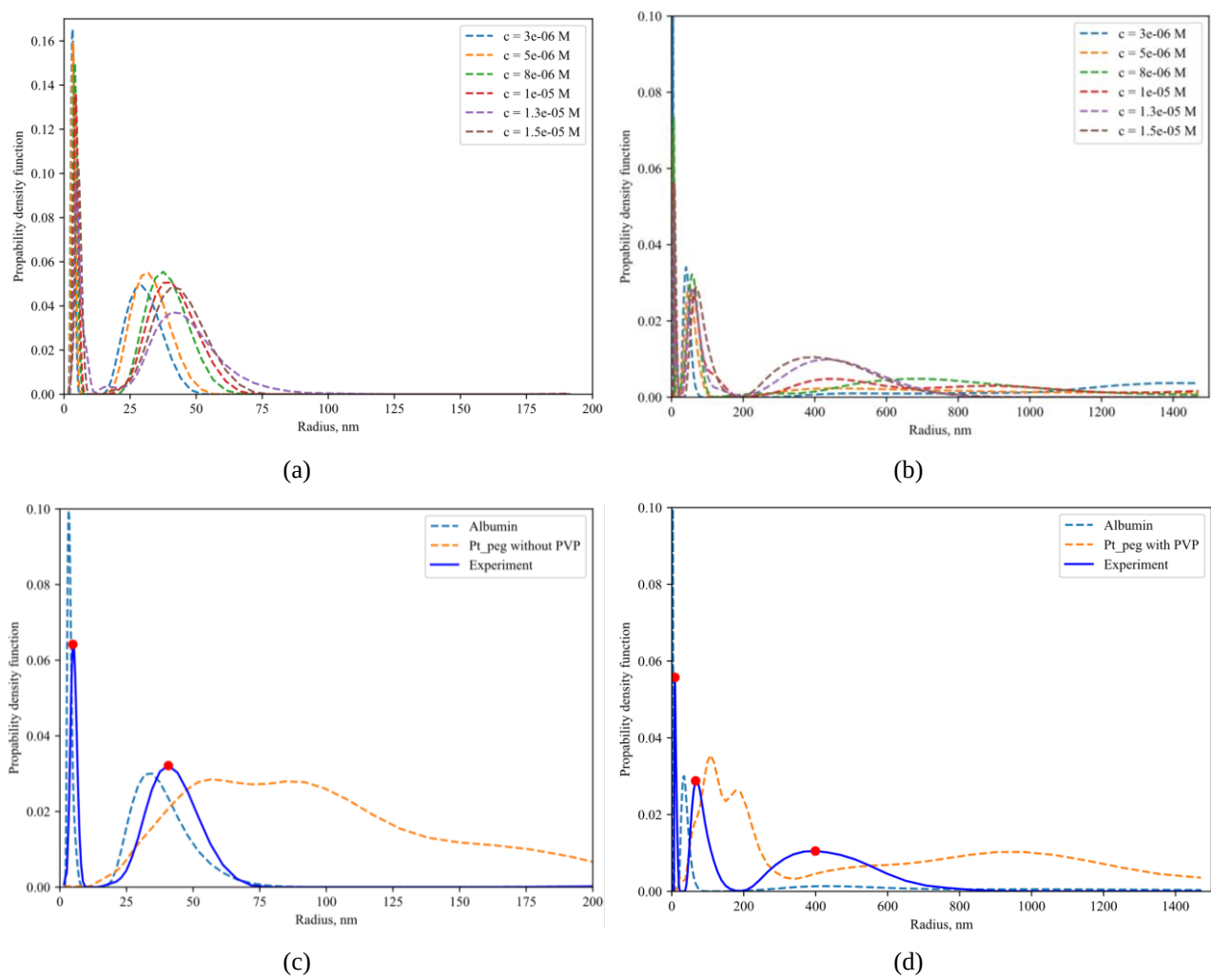


Fig. 5 Hydrodynamic radius determination for PT_peg with HSA: (a) Probability densities for corresponding size of aqueous solution of PT_peg and HSA. (b) Probability densities for corresponding size of aqueous solution of PT_peg_PVP and HSA. The red dots are the extremum points of the distribution density function.

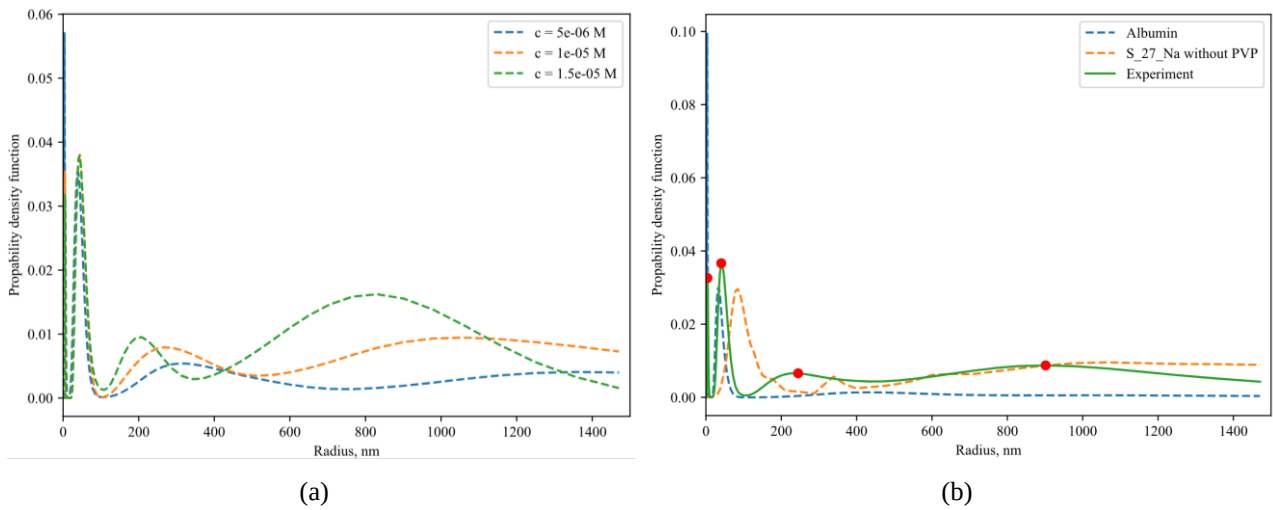


Fig. 6 Hydrodynamic radius determination for S_27_na with HSA: (a) Probability densities for corresponding size of aqueous solution (S_27_Na and HAS). (b) Comparison of probability density functions for corresponding sizes separately for HSA, S_27_Na, and solution. The red dots are the extremum points of the distribution density function.

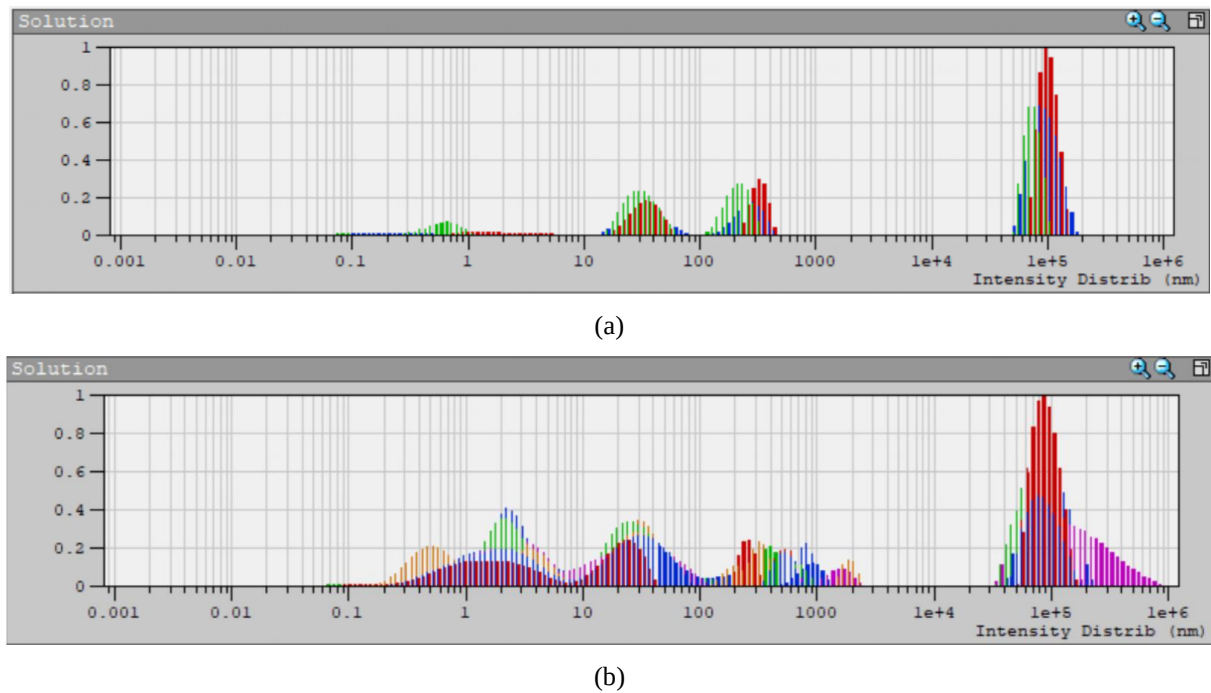


Fig. 7 (a) Peak distribution for S27_Na with PVP without HAS. DynaLS Software. (b) Peak distribution for S27_Na with PVP with HSA (Photosensitizer/Albumin Ratio = 1:1). DynaLS Software. Colors determine individual accumulations of correlation functions.

Table 3 Summary sample statistics.

Sample	Hydrodynamic radius, nm	Presence of a new peak in solution with albumin	PS interaction with albumin
HSA	3.6 ± 0.9 36 ± 10	—	—
Pt_peg	45 ± 14 112 ± 37	Absence	Absence of interaction
Pt_peg_PVP	90 ± 28 197 ± 40	New peak (400 nm)	Presence of interaction
S27_Na	92 ± 38 336 ± 24	New peak (215 nm)	Presence of interaction
S27_Na_PVP	62 ± 23 162 ± 36 339 ± 52	Multiple new peaks	Presence of interaction

4 Conclusions

In this way, all observed sizes were obtained in the study of aqueous solutions of PS with HSA (Table 3). The work demonstrated the effectiveness of the statistical approach in data processing when working with multicomponent biological media in comparison with the use of basic software. It was found that the Pt_peg type sample has the most stable state in the presence of transport HSA, whereas the S27_Na sample tends to interact with the protein. Additionally, the presence of PVP in the solution promotes a more pronounced interaction of HSA particles with the corresponding NPs.

As a result of a comparative analysis of self-assembled porphyrazine nanoparticles with and without PVP, properties were identified that make it possible to implement various methods of targeted drug delivery to tumor tissues. The DLS method revealed characteristic patterns in the interaction, or its complete absence, of the studied photosensitizer nanoparticles with HSA. Further research is focused on studying the process kinetics, in particular the binding constants and other parameters of the observed interaction.

Disclosures

The authors declare that they have no conflict of interest.

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Interrelation of Blood Microrheological Parameters Measured by Optical Methods and Whole Blood Viscosity in Patients Suffering from Blood Disorders: a Pilot Study

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Abstract. The perfusion of tissues and blood flux strongly depend on several interdependent parameters, such as viscosity of blood, red blood cell aggregation and red blood cell deformation. In case of many blood disorders these parameters are altered. This pilot study aimed to investigate the relationship between whole blood viscosity and microrheological parameters in patients with essential thrombocythemia (ET), as well as to compare microrheological parameters in patients with ET, erythrocytosis and spherocytosis. Blood viscosity measurements were performed using a rotational viscometer, while microrheological parameters were assessed through laser aggregometry and laser diffractometry techniques. The findings revealed novel data of the correlation between whole blood viscosity and microrheological parameters of patients with ET. Overall, this study provides valuable preliminary evidence on the relationship between whole blood viscosity and microrheological parameters in the context of blood disorders. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: aggregation; red blood cell; deformation; viscosity; essential thrombocythemia; spherocytosis; erythrocytosis.

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1 Introduction

Blood plays a vital important role in supplying oxygen to body tissues and in transporting various substances throughout the body [1]. Therefore, the parameters characterizing blood flux reflect the quality of blood functionality. In general, there is a division between the interrelated macrorheological parameters, i.e., blood viscosity and blood flow velocity, and the microrheological parameters, i.e., red blood cell (RBC) aggregation and RBC deformability [2–4].

Blood consists of plasma and blood cells, i.e., RBCs, platelets, and leukocytes. Blood is a shear-

thinning fluid, which means that its viscosity diminishes as the shear rate increases [3]. At low shear rates ($<100 \text{ s}^{-1}$), blood viscosity is primarily affected by RBC aggregation, whereas at high shear rates ($>100 \text{ s}^{-1}$) by hematocrit, RBC deformability, and plasma viscosity. RBC aggregation is a reversible process of formation of stack of coins-like linear and more complex structures called *rouleaux* [5]. RBC deformation is also reversible and plays a crucial role in the microcirculation since the average diameter of many terminal capillaries is smaller than the linear size of the RBC [4].

Deviation of the parameters related to the blood viscosity, i.e., RBC aggregation and deformation from the normal state, may be a result of pathological processes in the organism or disease complications [6–9]. Abnormal RBC aggregation and deformation may influence thrombotic events by promoting hemolysis, thrombophilia, inflammation, and microvascular occlusion [10].

In this work, we investigated groups of patients suffering from essential thrombocythemia (ET), erythrocytosis (ERY) and spherocytosis (SPH). ET and ERY belong to the group of chronic myeloproliferative disorders (CMD). Overall, CMD involve overproduction of different blood cell types in the bone marrow [11, 12]. Conditions like polycythaemia vera and primary myelofibrosis fall under this category. Essential thrombocythemia (ET) is characterized by overproduction of platelets in the bone marrow, leading to increased clotting risk and serious complications [13]. ERY refers to conditions with elevated red blood cell counts. Primary ERY is polycythaemia vera, where the bone marrow produces too many RBCs. Secondary ERY occurs as a response to low oxygen or other pathological alterations [14]. SPH is a hereditary condition characterized by the presence of spherical-shaped RBCs, which are more prone to destruction and can lead to anemia [15].

Haemostatic complications such as vascular thrombosis and haemorrhages significantly reduce the life duration of patients with CMD [16]. These patients commonly experience arterial or venous thrombosis, with arterial thrombosis being responsible for 60%–70% of events associated with myeloproliferative neoplasms [17, 18]. This type of thrombosis includes ischemic stroke, acute myocardial infarction, and peripheral arterial occlusion [18]. Numerous studies have investigated platelet aggregation, platelet function, bleeding [19–22], and cardiac complications associated with CMD [23]. However, research on the aggregation and deformation of RBCs in CMD patients has been comparatively limited, with less focus on this aspect compared to platelet aggregation. It was shown that the deformability of RBC is decreased, and the RBC aggregation is increased for patients suffering from polycythemia vera compared to the control group [24]. Also, the viscosity of whole blood is increased in polycythemia vera disease both for high and low shear rates [25]. Regarding the SPH, it was shown that deformability of RBC is decreased [26].

Table 1 Parameters of the studied groups.

Group	Number	Number of males	Number of females	Mean age ± SD
ET	11	8	3	11.3 ± 4.3
ERY	3	2	1	8.3 ± 4.5
SPH	4	1	3	12.3 ± 3.9

The goal of this work is to investigate for the first time the relationship between whole blood viscosity and microrheological parameters in patients with ET as well as to compare the microrheological parameters in patients with ET, ERY, and SPH.

2 Materials and Methods

2.1 Patients and Blood Samples Preparation

Overall, 18 patients with different blood disorders were enrolled in the study. The patients were divided into 3 groups based on their clinical diagnosis: ET group, ERY group, and SPH group. Parameters of the studied groups (mean age and number N) are presented in Table 1.

Blood samples were collected on an empty stomach from the patient’s cubital vein into 2 ml tubes with EDTA K2 or EDTA K3 anticoagulants. All patients were informed of the purpose of the research and gave the informed consent in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Medical Research and Educational Center of M.V. Lomonosov Moscow State University (protocol No. 11/22 from 05.12.2022). The experiments were performed within 6 h after blood collection, at which time the aggregation and deformation properties of RBC remain relatively stable [27]. In this paper, we do not consider a control group of healthy donors.

2.2 Viscosimetry

The rotational viscometer Lamy Rheology RM100 CP1000 (Lamy Rheology Instruments, France) acquired in the frame of Moscow State University Development Program equipped with MK-CP40Z measuring systems was used to measure the viscosity of whole blood. All measurements were performed under the room temperature ($T = 22\text{ }^{\circ}\text{C}$). For all experiments, shear rate of 1000 s^{-1} was set to measure blood viscosity. The viscosity at this high shear stress is primarily a function of hematocrit, plasma viscosity, and RBC deformability [28]. Blood viscosity is strongly dependent on hematocrit, so we further normalized blood viscosity from different samples using the constitutive models of Krieger and Daugherty [29]. This model is often used to obtain effective viscosity of blood and estimate the optimal hematocrit [30, 31]. The Krieger and Dougherty model states the following Eq.:

$$\eta = \eta_0 \frac{1}{\left(1 - \frac{\varphi}{\varphi_m}\right)^{2.5\varphi_m}}, \quad (1)$$

where φ_m is the max normalized volume of RBC in a suspension of plasma, φ is the current normalized volume of RBC in a suspension. For example, spheres can be packed with a maximum volume fraction of $\varphi_m \approx 0.74$. As far as RBC can deform the φ_m value is near the top limit ($\varphi_m \approx 1$). Thus, $\frac{\varphi}{\varphi_m}$ equals to the hematocrit. From Eq. (1), η_0 was calculated which corresponds to the theoretical viscosity of blood plasma. This calculated value is an additional parameter to normalize the whole blood viscosity by hematocrit and may not reflect the real plasma viscosity.

2.3 Laser Aggregometry

RBC aggregation was quantified using the diffuse light scattering technique implemented in the RheoScan-AnD300 device (RheoMediTech, Republic of Korea) [32, 33]. This method analyzes the scattered light obtained from a whole blood sample when illuminated by a laser beam ($\lambda = 635 \text{ nm}$, $P = 1.5 \text{ mW}$). Two types of disposable test chips were used to measure hydrodynamic strength of RBC aggregates and parameters of spontaneous RBC aggregation.

For performing the measurements of RBC hydrodynamic strength, a whole blood sample is placed into the reservoir connected to another reservoir by a thin

microchannel ($\approx 200 \mu\text{m}$) and the back-scattered light intensity is registered as the blood with continuously reduced velocity passes through (Fig. 1a). At high shear stress, RBC aggregates break up into smaller aggregates or single RBCs. When the shear stress decreases, the process of spontaneous aggregation of RBCs prevails. According to light scattering theory, the larger the particle size, the more light is scattered at small angles, and the forward scattering/backscattering ratio increases [34]. Thus, the changes of backscattered light intensity correspond to the changes in the mean size of RBC aggregates in the microchannel. The point of the maximum backscattered light intensity represents the balance between aggregation and disaggregation processes and is known as critical shear stress (CSS), which characterizes the hydrodynamic strength of RBC aggregates. The critical time (T_{crit}) refers to the specific moment when there is a balance achieved between RBC aggregation and RBC disaggregation (Fig. 1a).

The RBC aggregation kinetics is measured by registering the forward scattering intensity (Fig. 1b). In this case, the whole blood is placed in the microchamber, and the iron rod begins to rotate, resulting in complete disaggregation of the blood. When the rod stops, the kinetics of RBC spontaneous aggregation is measured and several parameters are calculated: the aggregation index (AI), characteristic time of RBC aggregation ($T_{1/2}$), AMP parameter, and M parameter [35, 36].

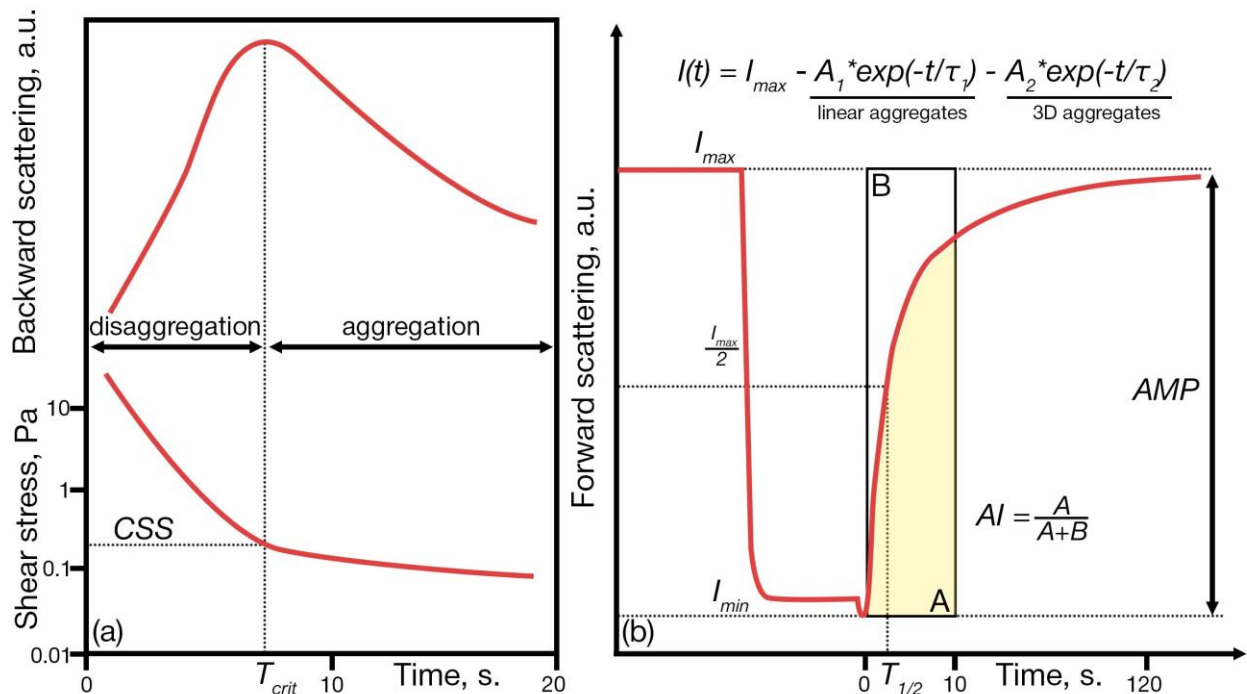


Fig. 1 (a) (below) The shear stress in the microchannel as a function of time; (above) the intensity of backward scattered light from the blood in the microchannel as a function of time. (b) The forward scattered light intensity ($I(t)$) from the whole blood sample in the chamber as a function of time (t) during RBC spontaneous aggregation. $I(t)$ is approximated by two exponential functions and linear aggregation (A_1, τ_1) and 3D aggregation (A_2, τ_2) parameters are calculated.

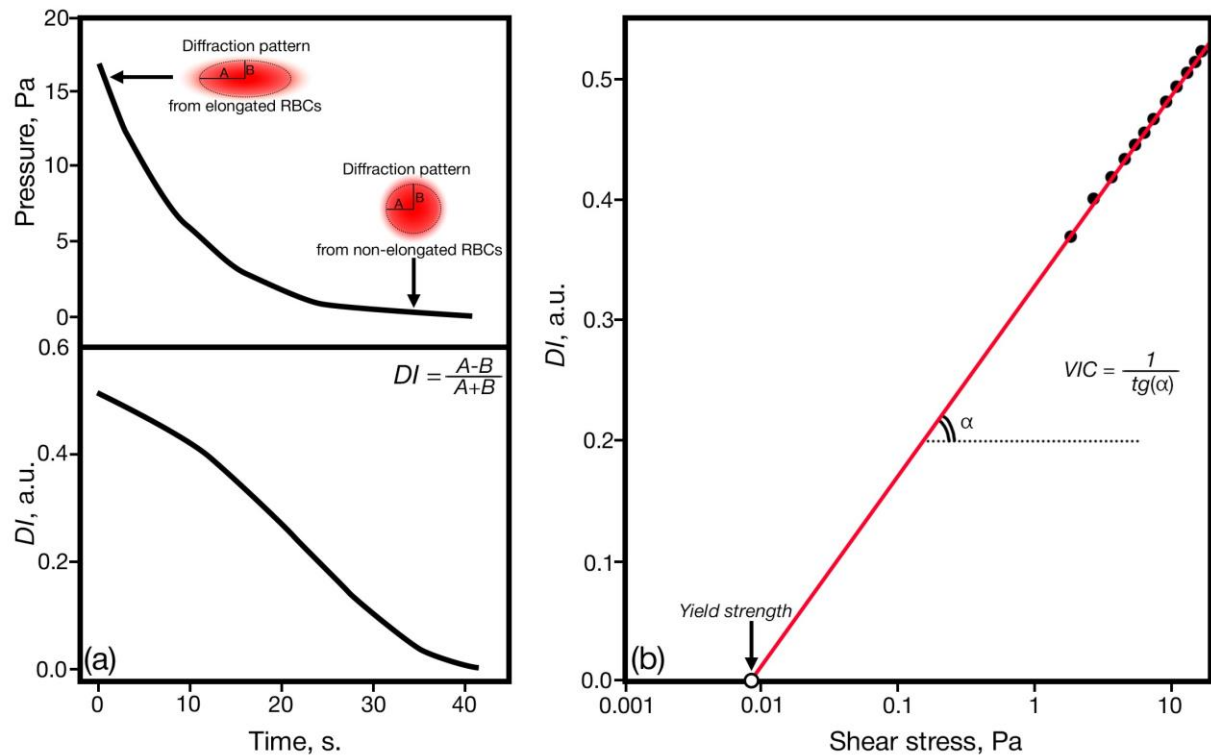


Fig. 2 (a) (below) The deformability index (DI) as a function of time (DI correspond to the relative elongation of RBCs); (above) the pressure difference at the ends of the microchannel in the microchamber as a function of time. (b) DI as a function of shear stress in semi logarithmic axes. The explanation of yield *Yield strength*, i.e., the minimum shear stress required for RBCs elongation, and RBC intracellular viscosity (VIC) calculation. α is the angle of slope of the curve.

In detail, AI characterizes RBC spontaneous aggregation in the first 10 s as well as M parameter equals to the area A (Fig. 1b). AMP indicates the difference in scattered light between the non-aggregated RBC state and the fully aggregated RBC state. $T_{1/2}$ characterizes the time to reach half of the maximum intensity. The more the RBC aggregation, the higher the values of parameters AI , M , and AMP and the lower the value of the $T_{1/2}$ parameter.

The process of RBC aggregation is not a gradual process, i.e., linear and 3D aggregation occurs simultaneously. However, by utilizing approximation methods, it is possible to extract parameters that correspond to either linear or 3D aggregation. Additionally, the curve ($t > 0$) was fitted using biexponential curve (see Eq. in Fig. 1b). The τ_1 corresponds to the characteristic time of linear RBC aggregates formation, whilst the τ_2 corresponds to the characteristic time of 3-dimensional (3D) RBC aggregates formation.

2.4 Laser Diffraction

RBC deformation was measured using laser ektacytometry technique which is also implemented in the RheoScan device (RheoMediTech, Republic of Korea) [37]. This method analyzes the diffraction patterns obtained from a diluted RBC suspension when illuminated by a laser beam ($\lambda = 635$ nm, $P = 1.5$ mW). As the RBCs elongate due to shear stress arising as a result of pressure difference at the ends of the microchannel, the diffraction pattern from the

cells also elongates, allowing for the calculation of the deformation index (DI) (see Fig. 2a). This provides a quantitative measure of the cells' ability to deform under different shear stresses.

Additionally, the relationship between DI and shear stress in semi-log scale was approximated using linear regression, allowing for the calculation of *Yield strength* and viscosity of RBC intracellular content (VIC). The determination of these parameters was described by Firsov et al. [38]. The change in VIC can be estimated by the slope change in the DI vs. log of shear stress plot, while the *Yield strength* is determined by the intersection with the X-axis (see Fig. 2b). The *Yield strength* represents the minimum shear stress needed for RBC deformation, and the VIC corresponds to the viscosity of its content.

2.5 Statistical Analysis of the Data

Custom developed python applications were used to process and plot all the data. In the Results and Discussion Section, the box plots in the Figs. 3–5 show the first quartile (Q1) to the third quartile (Q3) of the data, with a median line inside. Each point in the figure corresponds to the average value for one patient of at least 3 measurements of AI , M , AMP , and $T_{1/2}$; at least 7 measurements of η and CSS . The whiskers represent the standard deviations with the mean values (hole white points) at the center. To assess the statistical significance of differences between sample groups, the Mann-Whitney U test was conducted. Two sample groups were

considered significantly different if the p -value was less than 0.05. To determine the correlations between different parameters, Spearman's rank correlation coefficient was used. A correlation is considered weak if the absolute value of the coefficient was between 0.3 and 0.5, and strong if it was greater than 0.5.

3 Results and Discussion

3.1 Blood Viscosity, RBC Aggregation, and Deformation

The comparison of the measured parameters between ET, ERY, and SPH groups is presented in Figs. 3–5. No statistically significant differences in whole blood viscosity (shear rate = 1000 s^{-1}) were observed between the studied groups (Fig. 3). Nevertheless, the mean viscosity values at high shear rate for each group surpass the standard (normal) range of 3.5–5.5 mPa·s, as determined by many scientific groups [1]. Also, statistically significantly elevated hematocrit was observed in blood of patients with ERY (see Fig. 4). This is entirely consistent with medical evidence that ERY has a high hematocrit and/or hemoglobin level.

In Fig. 5 the dependence of AI parameter for 3 studied groups is presented. The statistically significant differences were found between ET and SPH groups ($p < 0.05$). It means that the RBC aggregation is reduced in patients with SPH and at the same time the blood viscosity is increased at high shear rates. This phenomenon can be explained by the fact that the shape of RBC in SPH is different from that of a biconcave disk and they deform much less. This in turn affects the RBC aggregation.

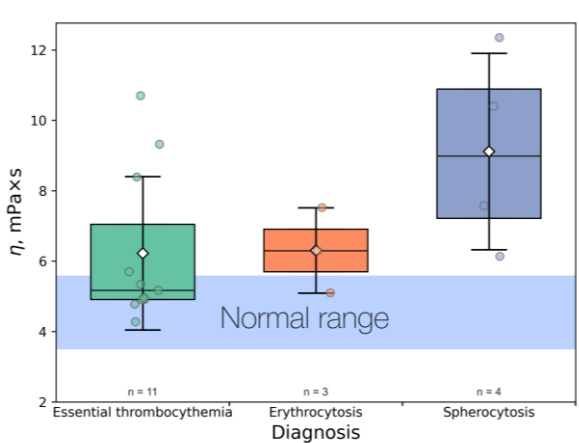


Fig. 3 Viscosity of whole blood (η) (shear rate = 1000 s^{-1}) for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. The normal viscosity range was determined from Ref. [1].

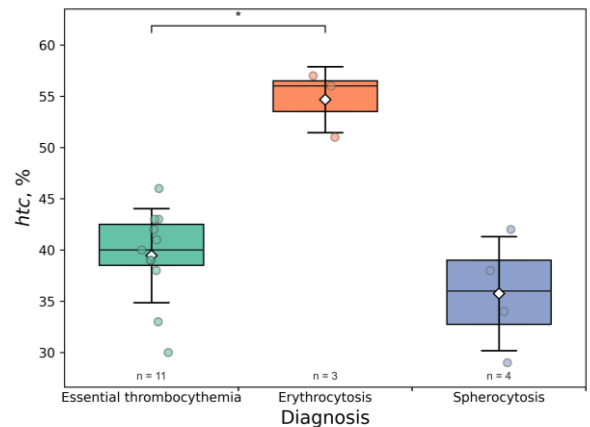


Fig. 4 Hematocrit (htc) for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. * $p < 0.05$.

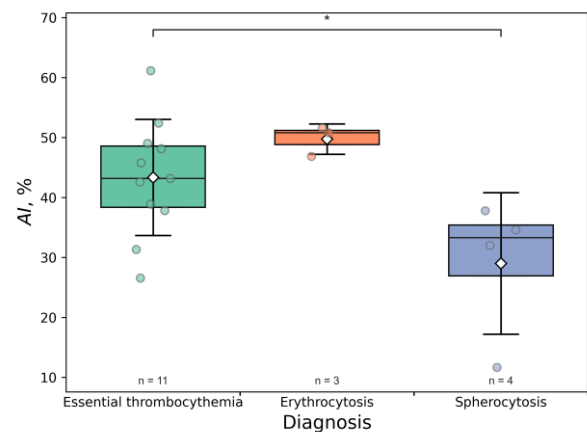


Fig. 5 AI parameter for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. * $p < 0.05$.

The other parameters characterizing RBC aggregation, i.e., M , AMP , $T_{1/2}$, τ_1 , τ_2 , CSS , were not statistically significantly different between the studied group. The further research is required to increase the number of participants and to add a control group of healthy volunteers in the study.

The deformability of RBCs statistically significantly decreased for the SPH group. This is most likely due to the altered RBC shape in patients with SPH. The deformability of RBCs in the blood of patients with ERY is slightly lower than that of patients with ET but is not statistically significant. Further studies are required.

Table 2 Parameters of RBC deformability for the studied groups. Means $\pm$ SD are presented.

Group	DI (3 Pa), a. u.	DI (20 Pa), a. u.	Yield strength, Pa	VIC, a. u.
ET	0.30 $\pm$ 0.03	0.48 $\pm$ 0.02	0.19 $\pm$ 0.05	4.1 $\pm$ 0.1
ERY	0.28 $\pm$ 0.07	0.45 $\pm$ 0.07	0.28 $\pm$ 0.23	4.2 $\pm$ 0.2
SPH	0.25 $\pm$ 0.07	0.37 $\pm$ 0.08	0.16 $\pm$ 0.17	6.5 $\pm$ 2.1
Control from Refs.	0.32 $\pm$ 0.02 [39]	0.51 $\pm$ 0.01 [40]	–	–

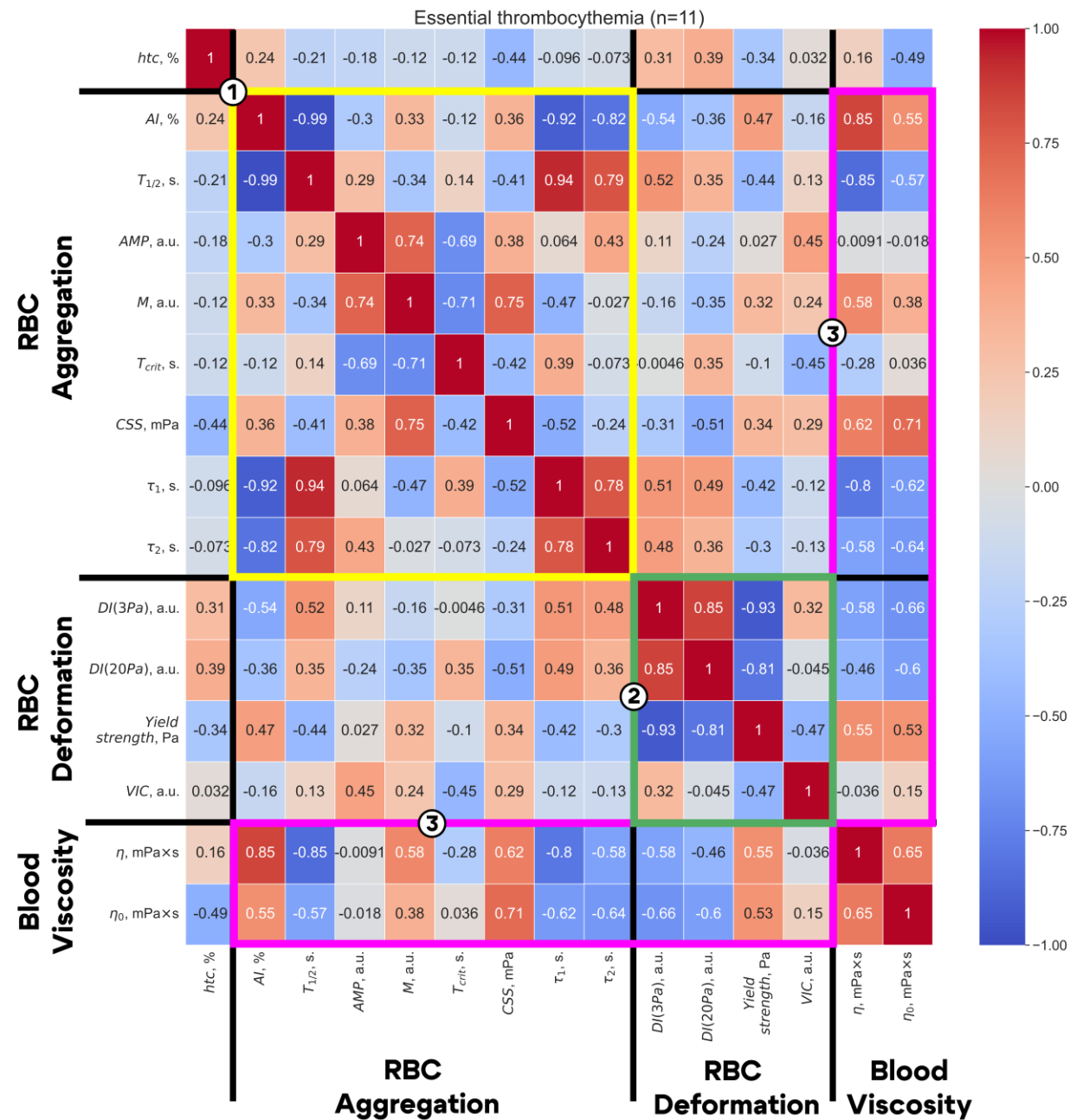


Fig. 6 Correlations between whole blood viscosity (η) (shear rate = 1000 s⁻¹), normalized viscosity (η_0), hematocrit (*htc*), and microrheological parameters for patients with ET. The correlations between parameters are characterized by the Spearman's rank correlation coefficient. The correlation matrix is divided by 3 groups of parameters, i.e., RBC aggregation, RBC deformation, and blood viscosity. Additionally, the correlations between RBC aggregation parameters are outlined by the yellow frame (#1); the correlations between RBC deformation parameters are outlined by the green frame (#2); the correlations between viscosity parameters and macrorheological parameters are outlined by magenta frames (#3).

Additionally, the mean values of the DI (3 Pa) and DI (20 Pa) parameters for all 3 groups are lower than in healthy adult subjects (see Refs. [39, 40]) (see Table 2).

Interestingly, the VIC parameter is elevated in patients with SPH. Although the work [26] demonstrated a lower spherocyte density, the increased VIC is likely due to the high intercellular heterogeneity of spherocytes.

The authors are not aware of any work that has investigated *Yield strength* and VIC using RheoScan. That is why the raw “Control from Refs.” from Table 2 is absent for the two last columns. It is incorrect to compare *Yield strength* and VIC measured with other ektacytometers [38], since these parameters depend on the geometry of the measuring cuvette.

3.2 Correlation between Viscosity and Microrheological Parameters for Patients with ET

In this section, only patients with ET were considered because of the low number of patients with ERY or SPH (see Table 1). The blood microcirculation in terminal capillaries and the blood flow in large vessels play a critical role in the functionality of the circulatory system. The apparent viscosity of whole blood is a key property that determines blood fluidity. In certain diseases, such as the ones shown in Fig. 3, the apparent viscosity of blood is elevated. To understand the underlying reasons for this increased viscosity, it is important to analyze the correlation between viscosity and microrheological properties of the blood. These microrheological properties, including deformability and aggregation of RBCs, are responsible for the intrinsic properties of blood. By establishing such correlations, we can gain insights into the factors contributing to alterations in blood flow. Additionally, these findings may have clinical implications, as modifying the microrheological parameters that affect blood flow could potentially help correct the elevated blood viscosity.

In Fig. 6, the Spearman's rank correlation coefficients between the whole blood viscosity and the microrheological parameters are presented for the patients with ET. One can see that there are certain differences for the correlation coefficients between RBC aggregation and RBC deformation properties, viscosity and microrheological properties, etc. Interestingly, the whole blood viscosity at the high shear rate (1000 s^{-1}) positively correlate with the AI parameter ($r = 0.85$) and negatively correlate with time parameters of RBC aggregation ($T_{1/2}$, τ_1 , τ_2) ($r < -0.55$). Also, negative correlation between blood viscosity and RBC deformation can be observed via Spearman's rank correlation coefficients (Fig. 6). This means that the lower the deformability of RBCs, the higher the blood viscosity, which is consistent with the general concept [1].

The weak positive correlation between CSS and AI parameters is observed ($p = 0.36$), whilst the high positive correlation between CSS and M parameters is observed ($p = 0.75$) (Fig. 6). It means that in patients with

ET, the higher the aggregation, the higher the hydrodynamic strength of the RBC aggregates. In other words, it means that the processes of RBC aggregation and disaggregation are positively correlated with each other. It should be noted that the M and AI parameters are calculated almost in the same way, except that the AI parameter is normalized, and the M parameter is not (Fig. 1). However, considering correlation matrix, one can see that M and AI parameters are not correlated ($p = -0.12$).

The parameter of normalized whole blood viscosity (η_0) is correlated with microrheological parameters almost the same as the whole blood viscosity (η). This probably means that plasma composition is a major factor affecting changes in whole blood viscosity at high shear stresses in patients with ET.

In the future, with large statistical magnification such correlation analysis between the parameters could be used in diagnosis or therapy monitoring. In this pilot study, due to the limitations imposed by the small sample group sizes, it is not feasible to draw broad conclusions.

This study has several limitations. First, there is no control group due to the lack of healthy people of approximately the same age as the patient. However, the results obtained from the small sample group size still provide preliminary conclusions on the relationships between whole blood viscosity and microrheological parameters for patients with ET. It is evident that the inclusion of more data from larger study groups will help reduce statistical scattering and yield more accurate information on the correlations being studied. Additionally, it is important to note that only patients aged 8–17 participated in this study. Increasing the number of individuals in the study groups will allow for the identification of any alterations in the studied parameters and their correlations, considering such factors as the age and the specific diseases being studied.

4 Conclusions

This pilot study has shed light on the correlation between whole blood viscosity and microrheological parameters in the context of blood disorders. The novel data presented have provided valuable preliminary evidence for better understanding the relationship between blood viscosity and microrheological parameters of patients with ET as well as between different microrheological parameters of patients with ET, ERY, and SPH. However, it is important to consider the limitations of this study, such as the small sample group size and lack of control groups. Therefore, further research with larger sample group sizes and control groups is necessary to validate these findings and determine the potential diagnostic and treatment implications for myeloproliferative disorders and SPH.

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Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Differential Rapid Diagnosis of Endometrial Cancer and Its Benign Pathological Conditions Using Surface-Enhanced Raman Spectroscopy

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Abstract. The purpose of this study is to improve the efficiency of early diagnosis of endometrial cancer using the analysis of surface-enhanced Raman scattering (SERS) of blood plasma. Blood plasma of patients aged 22 to 79 years was investigated. The study included 95 women. All patients were divided into 3 groups: group 1 consists of 29 women with endometrial adenocarcinoma, group 2 – 31 patients with endometrial polyp, group 3 – 10 women with endometrial hyperplasia. A control group consisted of 25 healthy women. The SERS spectra of dried samples were studied on an experimental stand consisting of a Photon-Bio RL785 spectrometric system based on a charge-coupled device (CCD) detector and a laser radiation source with a wavelength of 785 nm and an ADF U300 microscope. In order to realize the effect of surface enhancement of the Raman signal from blood plasma, we used a silver substrate based on a dried silver colloid. In the result of the study, the spectral features and specific features which characteristic of adenocarcinoma, polyps and endometrial hyperplasia were determined. With the use of discriminant analysis by projection onto latent structures (PLS-DA) method, the accuracy of optical diagnostics of endometrial adenocarcinoma relative to the control group and endometrial hyperplasia for the calibration and verification sets of spectra was 87% and 85%, respectively. Class discrimination accuracy of the control group with respect to endometrial hyperplasia and adenocarcinoma was 85%, and endometrial hyperplasia relative to the control group and endometrial adenocarcinoma was 81% for the verification set of spectra. The study shows the possibility of using SERS for differential express diagnostics of endometrial cancer and its benign pathological conditions. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: blood plasma; polyp; hyperplasia; adenocarcinoma; surface-enhanced Raman spectroscopy; SERS; PLS-DA; projection onto latent structures; discriminant analysis.

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1 Introduction

The key step in the diagnosis of diseases of any organs of the female reproductive system, such as inflammatory, dystrophic, borderline, tumor and other diseases associated with tissue changes, is a histological (pathomorphological) study. The true correspondence to the diagnosis and the legitimacy of the conclusion depends on the subjective opinion and decision of the pathologist and are determined by the level, knowledge, work experience, etc. Clinical cases are often complex, controversial, questionable and cause difficulties in making a diagnosis, which requires third-party consultation by higher-level specialists. However, the fate of the patient often depends entirely on the pathologist.

The presence of a highly accurate and at the same time independent of the expert method of pathomorphological diagnostics, such as “optical biopsy” of tissues, would allow to avoid many difficulties and correctly determine the patient management plan.

Benign endometrial diseases and benign diseases of the uterus (endometrial polyps (PE), endometrial hyperplasia (HE)) are currently one of the most common pathologies among patients with gynecological diseases [1]. Often, they are considered as a background for further cell transformation, which is the beginning of an atypical process. Diagnosis of PE and HE is important for women planning pregnancy both naturally and in assisted reproductive technology (ART) programs. As a rule, obstetrician-gynecologists cure PE, HE before pregnancy. Patients with intrauterine pathology are not allowed to the ART program. Resection of PE, HE by curettage is accompanied by severe trauma to the endometrium, followed by a potential inflammatory process and a decrease in the structural and functional potential of the endometrium. Polypectomy under hysteroscopic guidance, dilation and curettage for diagnostic HE is accompanied by severe trauma to the endometrium, followed by a potential inflammatory process and a decrease in the structural and functional potential of the endometrium.

Among benign diseases of the endometrium, the most common pathology is PE: 21.7–27.3%. According to Ref. [2], PE are rare at a young age, but their number doubles to 45–53.8% during perimenopause and postmenopause. According to Ref. [3], the incidence of malignant PE is 3.57%. Research results show that the risk of malignancy is higher in postmenopausal patients and in patients with abnormal uterine bleeding [3]. HE without atypia is detected in 4–10% of patients and tends to recur [4]. According to Ref. [5], the overall incidence of HE was 144 cases per 100,000 women. At the same time, the incidence of HE without atypia most often occurs in patients aged 50 to 54 years (from 142 to 213 per 100,000 women), and HE with atypia most often occurs in patients aged 60 to 64 years (56 per 100,000 women) [6]. According to Kaprin et al. endometrial cancer ranks first among tumors of the female reproductive system from 2010 to 2020, and the incidence of endometrial cancer in the Russian

Federation increased by 28.79% [7]. It should be noted that cancer of the body of the uterus is on the 3rd place in terms of frequency of occurrence among all cancers in the female population and is 8.0% [7]. The peak incidence occurs at the age of 60 to 70 years, but according to the latest data, cancer of the uterine body “gets younger” and in 2.0–5.0% of cases is detected in patients younger than 40 years [8].

Pathological conditions of the endometrium often occur without any specific clinical picture, which makes it difficult to diagnose these conditions. The gold standard for diagnosing benign endometrial diseases is still hysteroscopy with Dilatation and Curettage (D&C) and subsequent histological examination. This diagnostic manipulation is highly invasive, while the degree of tissue traumatization largely depends on the qualification of the doctor. The quality of the histological conclusion also largely depends on the human factor, namely the volume of the endometrial tissue sample and the subjectivity of the assessment of histological specimens.

Discrepancies in histological findings occur even among expert-level pathologists, and immunohistochemical studies are expensive and not always possible [9]. According to Polyakova et al. 7% of the conclusions obtained as a result of diagnostic dilation and curettage are not informative, and a discrepancy in diagnoses occurs in 30.6–71.9% of patients [10]. Based on the fact that diagnostic curettage of the uterine cavity has insufficient diagnostic value, is highly invasive and associated with intra- and/or postoperative complications, experts recommend avoiding this diagnostic method in patients of reproductive age.

Thus, it is an expedient task to develop a new highly efficient and high-precision, minimally invasive and economically low-cost diagnostic method for early and preclinical differential diagnosis of cancer and benign diseases of the endometrium. This study was carried out jointly with experts from physical laboratories and subdivisions of the Institute of Solid State Physics RAS and Samara University. For the differential diagnosis of endometrial pathologies, we analyzed the surface-enhanced Raman scattering (SERS) spectra of the blood plasma of patients with various endometrial diseases and the comparison group. A multidimensional analysis of a large array of spectral data was performed using the method of the projections to latent structures discriminant analysis. It should be noted that the proposed diagnostic method is inexpensive, fast, highly accurate and minimally invasive.

There are studies showing the possibility of using volumetric and SERS for the diagnosis of malignant tumors [11–13]. In 2015, using Raman spectroscopy, a group of volunteers with an intact bladder and a group of patients with a confirmed diagnosis of bladder cancer were compared, the classification accuracy was

96% [14]. In 2021, Artemyev et al. conducted an analysis of prostate tissue with the allocation of two groups: benign hyperplasia and prostate cancer. The classification accuracy was from 80% to 100% [15]. Currently, a small number of studies have been published

that provide data on the study of endometrial tissues using Raman spectroscopy [16]. In the studies, the authors used laser radiation with a wavelength of 1064 nm. In this study, a laser source with a wavelength of 785 nm was chosen to study blood plasma. Patients were divided into 3 groups: 25 blood plasma samples in the comparison group, 29 samples of adenocarcinoma and 41 samples of benign endometrial diseases (31 blood plasma samples from patients with PE and 10 samples of HE). In previously published studies, the described diagnostic method was not used in the study of such nosological forms.

2 Materials and Methods

2.1 SERS Spectral Acquisition

Raman spectroscopy is a widely discussed and promising method of tissue morphological diagnostics. This type of optical spectroscopy is based on the interaction of laser radiation with matter and the detection of the optical response from matter in the form of Raman light scattering using matrix photodetectors. During the interaction of laser radiation and matter, spectral components appear in the spectrum of scattered light that are shifted relative to the frequency of the exciting laser radiation by the corresponding frequencies of intramolecular vibrations. These frequency shifts appear in the spectrum and unambiguously determine the chemical structure of the substance.

Registration and analysis of the results of SERS of blood plasma were carried out using silver nanoparticles and a microscopic system. The experimental stand diagram is shown in Fig. 1.

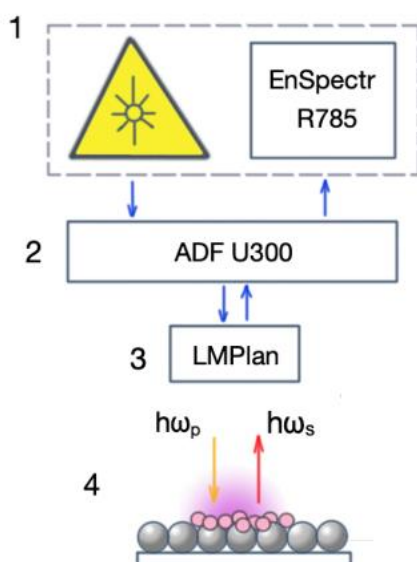


Fig. 1 Experimental setup: 1 – spectrometric system with laser module (785 nm central wavelength); 2 – microscope; 3 – objective; 4 – plasma sample on the silver substrate.

The study was made of the micro-Raman spectral characteristics with surface enhancement for plasma samples. The analysis of the spectral characteristics of the analyzed SERS substrate was carried out on an experimental stand consisting of a spectrometric system RL785 (LLC FOTON-BIO, Russia) based on a charge-coupled device (CCD) detector and a laser source with a wavelength of 785 nm and an ADF U300 microscope (ADF, China). An LMPlan objective with a magnification of 50× was used to focus the radiation on the sample and collect scattered radiation. The laser spot diameter at the focus was 5 μm. The spectra were recorded in the spectral range 380–1800 cm⁻¹ with a spectral resolution of 6–8 cm⁻¹. The limit of the permissible relative mean square deviation of wavenumber measurements was no more than 1%. The laser radiation power was 20 mW. The parameters for recording the spectra were as follows: the exposure time was 2 s, the number of averagings was 10. The spectra were recorded using the EnSpectr program. Immediately before recording the spectral characteristics of the studied plasma sample, a preliminary recording of the ambient background signal was made. After that, the background component was automatically subtracted from subsequent recorded spectra of the sample.

2.2 Colloidal Silver Nanoparticles Solution

A silver substrate based on a dried solution of colloidal silver was used as a substrate material to achieve the effect of SERS of the plasma [17]. A colloidal solution of silver was obtained by reduction from an aqueous solution of silver nitrate with sodium citrate at a temperature of 95 °C for 20 min. The absorption spectrum of the obtained colloidal silver solution has an absorption maximum at 410 nm with a half-width of 40 nm, which, according to a number of experimental studies of the dependence of the absorption spectrum on the geometric characteristics of silver nanoparticles, corresponds to spherical nanoparticles with a diameter of 30–40 nm. To form more complex and large structures, the resulting colloidal solution with a volume of 20 ml is applied to aluminum foil with an area of 75 mm × 25 mm and dried at room temperature until completely dry. As a result of drying, agglomerates of silver particles are formed on the foil. The resulting substrate is agglomerates of spherical particles with a size of about 200 nm.

The characteristics of the substrate are presented in more detail in Ref. [17]. It should be emphasized that an important feature of the approach to spectral analysis used is the insignificant spectral contribution of the silver substrate to the spectrum of the tested sample, which simplifies the subsequent analysis due to the absence of the need to take into account the substrate spectral contribution in the analysis. Integration of spectral characteristics from a sample region of several microns within 2 s provides a number of scattering events sufficient to level out blinking acts. Analysis of substrate stability demonstrated that the blinking effect is not

significant, the standard deviation of the sample spectral characteristics on a substrate is no more than 8% [17].

2.3 Plasma Samples Preparation

Not all available samples were included in spectral studies, since many of them were not intended for this due to blood hemolysis (violation of the biomaterial sampling technique and individual characteristics of patients' hemostasis), small sample volume, or thick consistency. As a result, the following groups were formed for the SERS analysis of blood plasma: the control group (25 blood plasma samples), PE (31 plasma samples) and HE (10 samples), which were combined into one group, and endometrial adenocarcinoma (29 plasma samples).

Spectral studies included 95 blood plasma samples taken from patients aged 22 to 79 years. All women gave written informed consent to participate in the study. To confirm the diagnosis, a histological examination of endometrial tissues was performed. These tissues were obtained during dilation and curettage, or tissue excision was performed immediately after hysterectomy. In the control group, we included 25 patients aged 22 to 40 years without clinical and laboratory signs of endometrial pathology. Histological confirmation of the absence of endometrial pathology became possible because of diagnostic dilation and curettage due to a cervical canal polyp in 19 patients (76%) and primary infertility in 6 patients (24%). In the second group, we included 10 patients aged 28 to 63 years with a histologically confirmed diagnosis of HE and 31 patients aged 28 to 63 years with a histologically confirmed diagnosis of an PE. Among these patients, 12 (29%) patients came to the gynecologist with complaints of abnormal uterine bleeding, and in 29 (71%) patients, endometrial pathology was suspected according to the planned ultrasound of the pelvic organs. Pipelle endometrial sampling in an outpatient setting was performed in 10 patients, but histological confirmation of the presence of endometrial pathology was obtained only in 3 (30%). The third group included 29 patients aged 51 to 79 years with a confirmed diagnosis of highly differentiated endometrial adenocarcinoma, all women were postmenopausal. Ten (34%) patients were diagnosed with cancer *in situ*, and 19 (66%) patients had a tumor that invaded less than half the thickness of the myometrium (T1a). Twenty two (76%) patients complained of abnormal uterine bleeding, and in 7 (24%) patients, endometrial pathology was suspected during a planned ultrasound of the pelvic organs. It is worth noting that 15 out of 19 patients (79%) with tumor invasion into the myometrium complained of abnormal uterine bleeding. Patients included in the study groups did not take hormonal drugs for 12 months, they were not diagnosed with acute infectious diseases and exacerbations of chronic somatic diseases.

Before the introduction of intravenous anesthesia, blood in a volume of 4 ml was taken into centrifuge vacuum tubes for hematological studies with EDTA-K2. Within 30 min after sampling the biological fluid, blood

plasma was separated by centrifugation at 3500 rpm for 5 min. The resulting plasma in a volume of 1 ml was transferred into a sterile test tube without filler using an automatic dispenser and frozen at a temperature of -18°C . Subsequently, three Raman scattering spectra were recorded for each sample.

2.4 Spectra Processing

The main method for improving the performance of statistical models is the optimal choice of a strategy for preprocessing spectral data. The task of preprocessing is to isolate a component of value for further analysis by eliminating third-party components, noise, correcting baseline, and normalizing the signal [18]. Therefore, before applying the multivariate analysis, the recorded spectral data of the plasma were freed from noise by the Savitsky-Golay method [18], and from the background radiation by correcting the reference line and baseline using the baseline correction with asymmetric least squares (baseline ALS) method [19].

Preprocessed blood plasma spectra, characterized by multidimensionality and multicollinearity, were analyzed using the method of discriminant analysis by projection onto latent structures (PLS-DA). The combination of PLS-based multivariate analysis and SERS is a powerful analytical tool and demonstrates the stability and promise of human plasma and serum analysis to discriminate patients by typhoid stage [20], to classify patients by the presence of the absence of colorectal cancer and precancerous neoplasms [21], to discriminate patients according to the severity of renal failure [22]. The PLS-DA method is a common approach for the chemometric analysis of spectral data from serum and plasma.

In this study, each plasma sample corresponds to a priori information about belonging to a particular group. Therefore, the data was analyzed through supervised learning. Based on the recorded intensity of the Raman spectra, a matrix was compiled, where each sample was assigned a value of 1, 2 or 3, depending on the class: comparison group (1), HE and PE (2) or endometrial adenocarcinoma (3). The application of the SIMPLS [23] algorithm makes it possible to calculate the PLS factors directly as linear combinations of the original variables. The PLS factors are defined in such a way as to maximize the covariance criterion, subject to certain orthogonality and normalization constraints. To avoid overfitting, the assessment of the stability of the experimental data analysis method and the choice of optimal parameters were implemented on the basis of k-fold cross-validation ($k = 7$). Cross-validation is a widely used technique for evaluating the quality of a model when analyzing spectral data. The general procedure is to split the data into subsets for training and testing. Training is the process of fitting a model, and testing is the process of checking the fitted model by measuring the prediction error. The training and test sets do not overlap, so the test data for model evaluation is not used in model fitting.

In order to avoid overestimation of any of the studied groups by the model, it is necessary to provide an

analysis of equally sized samples. To do this, 21 spectra were randomly selected from the total data set for each analyzed group. Based on the selected data, a classification model was built. The process of selecting samples of equal size and then obtaining a model was thus repeated 5 times. The final characteristic of the classification was obtained on the basis of averaging the results for 5 models. Sensitivity (Eq. (1)), specificity (Eq. (2)), accuracy (Eq. (3)), and error curve (ROC curve) [24] were used as parameters characterizing the resulting classification model.

$$\text{Sensitivity} = \frac{TP}{TP+FN}, \quad (1)$$

$$\text{Specificity} = \frac{TN}{TN+FP}, \quad (2)$$

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN}, \quad (3)$$

where TP – the number of true positive results, FP – the number of false positive results, TN – the number of true negative results, FN – the number of false negative results.

The PLS-DA method made it possible to reveal the spectral features of the classes associated with the presence of compounds and molecules determined as markers of endometrial diseases. All preprocessing methods and multivariate analysis were implemented in the RStudio (version 2023.03.0+386 for Windows).

Raman peaks belonging to certain classes of substances were determined using the database of Raman spectra of biological tissues [25–27].

3 Results and Discussion

During the study, spectral patterns were obtained for the control group in comparison with the HE and PE group, and the endometrial adenocarcinoma group (Fig. 2).

When studying the averaged spectra of SERS of blood plasma, the following spectral bands were identified: 596 cm^{-1} (phosphatidylinositol), 640 cm^{-1} (C-S stretching and C-C twisting in tyrosine), 726 cm^{-1} (C-S vibrations in proteins, CH_2 , adenine, hypoxanthine, coenzyme A), 766 cm^{-1} (compression of the pyrimidine ring), 815 cm^{-1} (C-C-O stretching vibrations, L-serine, glutathione, proline, hydroxyproline, tyrosine, PO_2 stretching in nucleic acids), 891 cm^{-1} (C-O-H bending vibrations, saccharide, glutathione, D-galactosamine), 933 cm^{-1} (proline, hydroxyproline), 956 cm^{-1} (stretching vibrations C-C, alpha helix, proline, valine), 1527 cm^{-1} (carotenoid), 1004 cm^{-1} (phenylalanine), 1055 cm^{-1} (C-C lipid stretch, collagen), 1132 cm^{-1} (tyrosine, D-manose), 1208 cm^{-1} , 1265 cm^{-1} , and 1276 cm^{-1} (amide III, L-tryptophan, phenylalanine), 1314 cm^{-1} (CH_3 , CH_2 twisting in collagen, stretching vibrations, stretching vibrations of CH bases of nucleic acids), 1477 cm^{-1} (CH_2 , collagen, phospholipids, deoxyribose), 1617 cm^{-1} (tryptophan, phenylalanine, tyrosine).

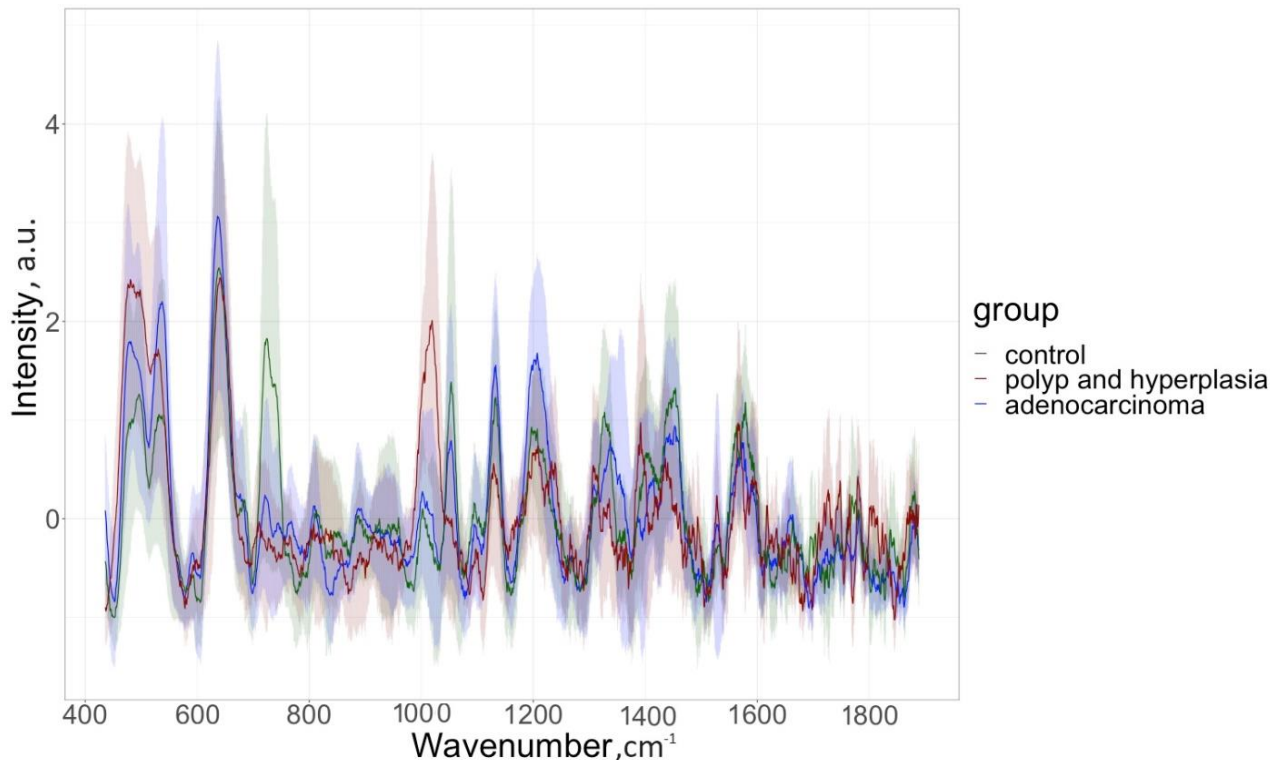


Fig. 2 Average processed SERS spectra of blood plasma from samples with endometrial hyperplasia and polyp, and endometrial adenocarcinoma in comparison with the control group. Filling with color indicates standard deviation.

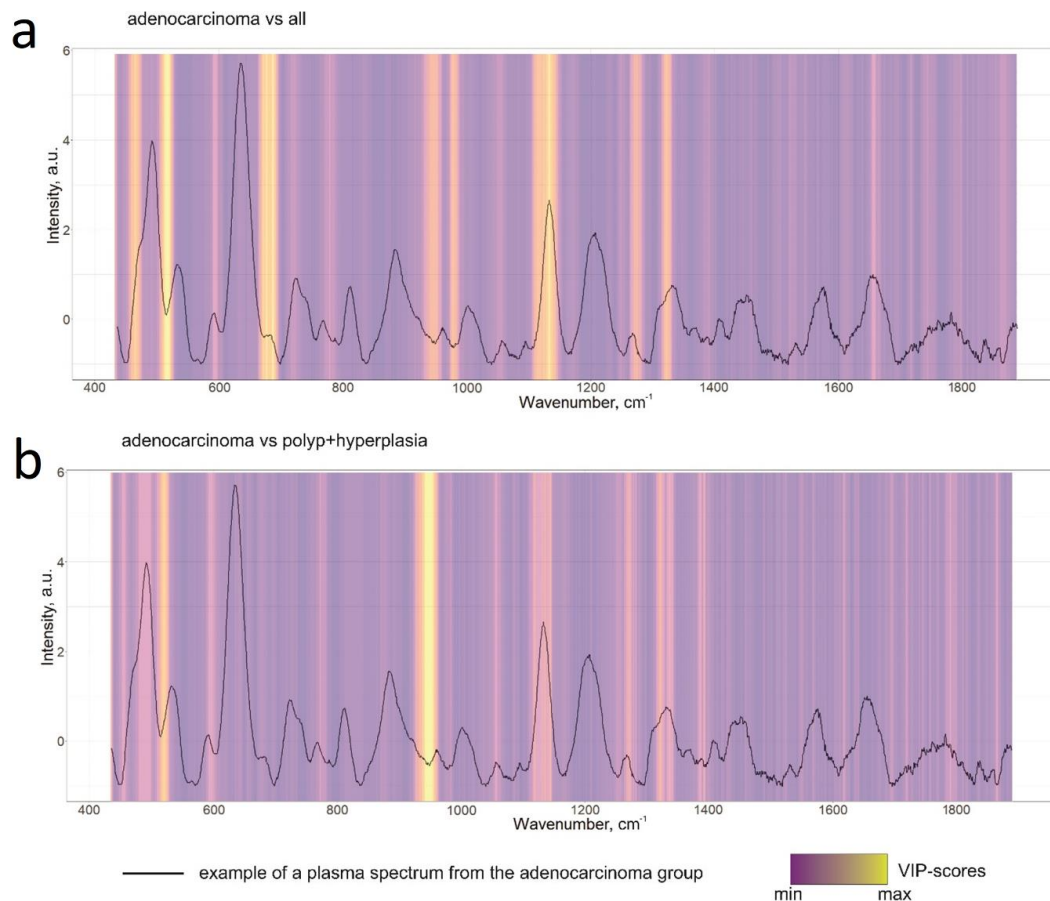


Fig. 3 The distribution of the variables importance for projection surface-enhanced Raman spectra of blood plasma for classifying samples according to a pathologically associated feature when implementing methods for analyzing experimental data based on PLS-DA. (a) Classes: control group, hyperplasia, adenocarcinoma, (b) classes: hyperplasia, adenocarcinoma.

Common spectral bands for PE and HE groups: 858 cm^{-1} (tyrosine, collagen), 917 cm^{-1} , and 974 cm^{-1} (stretching vibrations C-C: alpha helix, proline, valine), 1080 cm^{-1} (tensile vibrations C-N: collagen; phosphate vibrations: phosphodiester groups in nucleic acids) [28–32]. The band at 1242 cm^{-1} belongs to the group with PE; it is characteristic of the amide III vibration; for the other two groups, the band is absent. The band at 1418 cm^{-1} is the CH_2 oscillation [33] in lipids; it is typical for the group with polyps, for the control group it is shifted to 1416 cm^{-1} , and it is absent for the group with hyperplasia. The studied groups can also be detected not only by the shift of the bands, but also by the intensity, the most revealing were the bands at 465 cm^{-1} , 536 cm^{-1} , 816 cm^{-1} , 996 cm^{-1} , 1055 cm^{-1} , 1132 cm^{-1} , and 1208 cm^{-1} .

The distribution of the importance of variable SERS spectra of plasma (Fig. 3) when classifying samples according to a pathologically associated trait provides an opportunity to separate groups according to the most significant bands.

We have identified significant Raman peaks such as 724 cm^{-1} (C-H bending vibrations, adenine), 740 cm^{-1} (thymine, uracil), 1015 cm^{-1} (phenyl ring vibrations), 1055 cm^{-1} (proline), 1132 cm^{-1} (D-mannose), 1211 cm^{-1}

(phenyl ring vibrations), 1337 cm^{-1} (CH_2 vibrations of proline, purine bases of DNA), 1391 cm^{-1} (tryptophan), 1453 cm^{-1} (CH_2 bend in proteins), 1580 cm^{-1} (pyrimidine ring and hemoglobin proteins). The Raman peak at 724 cm^{-1} corresponds to the C-H bending vibrations of adenine, and the 740 cm^{-1} peak corresponds to nitrogenous bases such as thymine and uracil. According to studies, changes in the intensity of these peaks indicate an abnormal metabolism of DNA and RNA bases in blood serum [34]. Changes in the intensity of the 1015 cm^{-1} peak were also detected, these changes are of the greatest importance for the selection of a class of polyps, while the averaged spectra show an increase in the intensity of Raman scattering for polyps in this band. This may be related to the angular vibrations of the phenyl ring due to the higher content of phenylalanine [35]. A more intense value of the Raman peak at 1132 cm^{-1} , which indicates the content of D-mannose in the blood serum, may be associated with a violation of the processes of glycolysis and oxidation of pyruvic acid (Krebs cycle) [36]. The peak at 1211 cm^{-1} , as well as the peak at 1015 cm^{-1} , is probably associated with vibrations of the phenyl ring due to the higher content of phenylalanine.

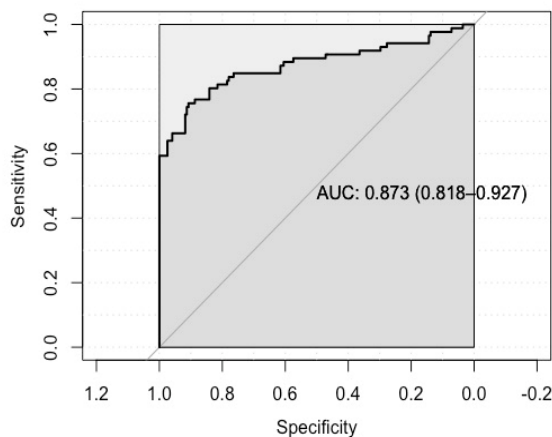


Fig. 4 The ROC curve of differentiation of endometrial adenocarcinoma relative to the control group and the group of HE+PE.

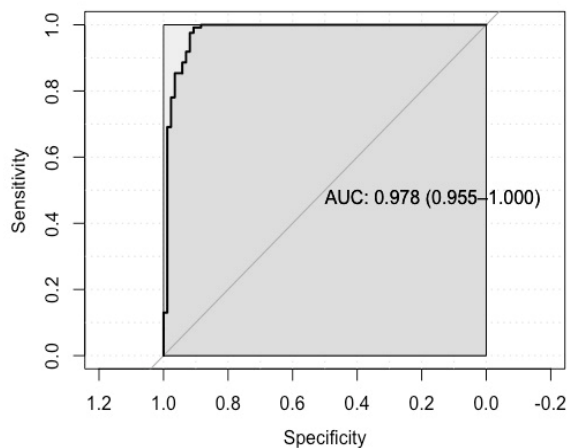


Fig. 5 The ROC curve of differentiation of endometrial adenocarcinoma relative to the group of HE+PE (without a control group).

As a result of building an analytical model using the PLS-DA method to further evaluate the effectiveness of the PLS-LDA classification model, ROC curves were obtained and differentiation was made between samples with adenocarcinoma and the other two groups – the control group and the hyperplasia group, which included PE (Fig. 4). The accuracy of differentiation of endometrial adenocarcinoma relative to the control group and the group of HE and PE for the calibration and validation datasets was 87% and 85%, respectively (66% sensitivity, 92% specificity for the validation set). The classification accuracy of the control group relative to the HE and PE group and the endometrial adenocarcinoma group was 86% and 85%, and the HE and PE group relative to the control group and the endometrial adenocarcinoma group was 81% for the calibration and validation datasets. At the same time, the accuracy of class discrimination of adenocarcinoma and HE (including PE) was 93% for the calibration data set (96% sensitivity, 90% specificity) and 91% for the

validation data set (93% sensitivity, 88% specificity) (see Fig. 5).

In 2020, a group of scientists conducted a study of endometrial pathologies using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). The study involved 652 women who were divided into 3 groups: endometrial cancer (342 samples), HE with atypical HE (68 samples), control (242 samples). The sensitivity and specificity of endometrial cancer detection by this method was 87% and 78%, respectively [37]. Based on the results of our study, we believe that the use of SERS is a more promising method for diagnosing endometrial cancer. Our findings are supported by a 2021 study in which endometrial structure was assessed using Fourier transform Raman spectroscopy and ATR-FTIR. The authors also concluded that Raman spectroscopy is more effective than FTIR in assessing the development of carcinogenesis in endometrial cancer [16].

Our research shows the ability to detect diseases and the potential of the SERS method to diagnose specific types of endometrial pathologies. In this study, the laser wavelength of 785 nm is optimal for the following reasons: (1) lower fluorescence from the samples and the SERS substrate (under potential excitation); (2) higher intensity of Raman scattering compared to the use of laser radiation with a wavelength of 1064 nm; (3) the possibility of using a cheaper silicon detector for 785 nm radiation, in contrast more expensive InGaAs detectors using laser radiation with a wavelength of 1064 nm. Due to the complex composition of blood, it is very difficult to distinguish the molecular species contributing to the overall SERS spectra, but there are certain cancer-associated metabolites that cause differences in the observed spectra of adenocarcinoma compared with normal tissue and hyperplasia. And SERS allows us to increase the signal-to-noise ratio of spectra, which leads to a reduction in registration time and an increase in the reliability of registration of spectral features.

4 Conclusion

In the present study, we have shown the potential value of using Raman spectroscopy in clinical practice for the differential diagnosis of benign and malignant pathologies of the endometrium. A literature review confirmed the absence of similar studies. The use of laser radiation with a wavelength of 785 nm in combination with SERS of blood plasma using a silver substrate showed a higher diagnostic efficiency.

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President of the Russian Federation dated 02/09/2009 No. 146 “On measures to strengthen state support for young Russian scientists - candidates and doctors of science” (grants of the President of the Russian Federation), agreement No. 075-15-2022-767 dated May 12, 2022 (MK-5445.2022 .1.2).

Disclosures

The authors declare that they have no conflict of interest.

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Discrimination of Optical Properties of Healthy and Cancerous Ovarian Tissue

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Abstract. In this work, the optical properties of ovarian tissue of cats with histologically confirmed diagnoses: leiomyosarcoma, stromal sarcoma, serous carcinoma, granulosa carcinoma, follicular phase (healthy) and luteal phase (healthy), in the optical range from 450 to 800 nm were experimentally studied. Based on the measured spectra of diffuse reflectance, total transmittance and collimated transmittance spectra, absorption and scattering coefficients were determined using the inverse adding-doubling method, as well as the spectral dependence of the scattering anisotropy factor and the depth of radiation penetration into ovarian tissue in normal conditions and in various types of cancer. The difference in the scattering and absorption properties of healthy and cancer ovaries can serve as an important and promising marker of pathology in the early stages in clinical applications, as well as in the search and prove of diagnostically significant criteria for assessing the metabolic state of tissues. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: ovarian of cats; leiomyosarcoma; stromal sarcoma; serous carcinoma; granulosa carcinoma; follicular phase; luteal phase; spectra of diffuse reflectance; spectra of total transmittance; collimated transmittance spectra; scattering anisotropy factor; absorption coefficient; scattering coefficient; the inverse adding-doubling method.

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1 Introduction

One of the pressing issues of modern medicine throughout the world is the development of the tumor diseases of various organs, including the ovaries, and the prevalence of tumors in the population. Ovarian cancer is one of the most common malignant diseases of the reproductive system among women of all age groups. More than 13 thousand new cases of malignant ovarian tumors are registered in Russia every year. The increase in the incidence of ovarian cancer over the past 10 years

has been 5.3%. About 70% of patients at the time of diagnosis of the disease have stages III–IV, which gives virtually no chance of survival [1]. Despite the advances in drug therapy for ovarian cancer and the appearance of platinum drugs and then taxanes in the early 80s, according to generalized data from population-based cancer registries in European countries, mortality in the first year of life of patients remains high and amounts to 63%, and the five-year survival rate is not more than 35%. Because of this, the world community annually suffers irreparable losses among the active female

population with powerful life potential. This disease is difficult to verify due to the lack of pathognomonic symptoms and timely diagnosis [2]. The development of new improved optical methods used in various fields of biology and medicine undoubtedly requires knowledge of the optical properties of tissues [3]. Such modern promising methods as photodynamic and photothermal therapy, endoscopic surgery, treatment using laser radiation, optical diagnostics of various physiological processes and pathologies, including oncology, require the development of mathematical models that adequately describe the propagation of light in tissues, for the development of safe techniques and widespread implementation into clinical practice [4]. One of the modern clinical technologies, actively used, in particular in gynecology, allowing for optical-endoscopic imaging and treatment, is laparoscopy. The optics of the laparoscope are inserted into the abdominal cavity through a small incision, which allows one to directly examine the pelvic and abdominal organs or, by connecting a video camera, transfer the image to the monitor [5, 6].

There is great interest in optical studies of the female reproductive system. For example, a hyperspectral light source has been developed, based on the chromatic dispersion property of ready-made lenses, which can be built into a standard endoscope/microscope to obtain hyperspectral images (label-free images) [7]. Optical coherence tomography (OCT) has been used to evaluate the ability to non-invasively characterize ovarian follicle developmental morphology and age-related changes in mice [8]. The combined use of photoacoustic and ultrasound imaging to assess vasculature and collagen content in the intact ovary has been discussed [9]. A new spatial frequency domain imaging (SFDI) technique is proposed for non-contact, rapid assessment of the optical properties of ovarian tissue over a large field of view [10]. The use of broadband spectral imaging with excitation of endogenous fluorescence by UV light with a wavelength of 365 nm made it possible to classify all image pixels using linear discriminant analysis and create diagnostic maps of the ovaries [11]. OCT has also been used to assess the scattering coefficient from unfixed normal and malignant ovarian tissue *ex vivo*, as well as to assess collagen distribution [12, 13]. Unfortunately, studies of the optical properties of ovarian tissue, both normal and pathological, discussed in the literature do not provide comprehensive data on determining the absorption and scattering coefficients and anisotropy factor. To validate optical measurement methods, tissue phantoms are often used which mimic and retain optical parameters over long period of time [14].

The optical properties of many tissues, in particular ovaries in normal conditions and in oncological pathologies, remain currently insufficiently studied in a wide range of wavelengths, although they are of fundamental importance for a deep understanding of the interaction of radiation with tissues in diagnostics and phototherapy. One of the most commonly used algorithms for solving the inverse optical problem for

reconstructing the reduced (transport) scattering and absorption coefficients of turbid media is the inverse adding-doubling (IAD) method. It was developed by Prahl and is used to determine the optical parameters of many tissues [15]. The IAD method is well suited for calculations using diffuse reflectance and transmittance spectroscopy data. The IAD method was used to study the optical properties of the human lens with different stages of cataracts [16], peritoneal tissue in the spectral range of 350–2500 nm [17], normal tissues of the human colon [18] and in comparison with polypous changes [19], human gingival tissue and dentin [20], as well as other tissues (skin, muscles, skull, etc.) [3, 4, 21, 22].

The goal of this study is to determine and compare the optical properties of healthy and cancerous cat ovaries, namely absorption coefficient (μ_a), scattering coefficient (μ_s), and anisotropy factor (g), as well as the depth of light penetration into tissue in the range from 400 to 800 nm.

2 Materials and Methods

In the *ex vivo* work, we examined the ovaries of outbred cats aged 5 to 12 years, obtained after ovariectomy and ovariohysterectomy in a veterinary hospital. To clarify the diagnosis, we performed a histological examination of the samples no later than 48 h after removal. To do this, a portion of the sample was manually excised from each ovary with a scalpel and fixed in 10% buffered formalin. The remaining parts of the ovaries were kept frozen until optical measurements were performed. Histological studies were carried out in specialized laboratories. The thickness of tissue sections (samples placed between glass slides) was measured with an electron micrometer (Union Source CO., Ltd., China, Ningbo) at several points on the sample and then averaged. The accuracy of each measurement was ± 0.01 mm.

In total 84 samples (tissue sections) were examined. To clarify diagnoses, 18 sections (3 from each of 6 ovary types) were used for histological studies. To measure the refractive index 18 sections (3 from each of 6 ovary types) and for spectral measurements (see Table 1), 48 sections (5 and 3 from each of 6 ovary types for DRS/TTS measurements and collimated transmission, respectively) were used.

To measure the total transmittance and diffuse reflectance of tissue samples in the spectral range of 450–800 nm, a Shimadzu UV-2550 dual-beam spectrophotometer (Japan) with an integrating sphere was used. The radiation source was a halogen lamp with radiation filtering in the spectral range under study. The maximum resolution of the spectrometer was 0.1 nm. The spectra were normalized before measurements using a reference reflector BaSO₄, which has appropriate properties in the UV [23]. All measurements were carried out at room temperature (~ 25 °C) and normal atmospheric pressure.

Table 1 Number of tissue sections n for each ovary type and their properties.

Ovary	Ovary type (Histology, $n=3$)	Number of ovaries (Spectral study, $n=5$)	Mean thickness $\pm$ SD, mm	Refractive index (589 nm), $n=3$
Healthy	Follicular phase	5	0.80 ± 0.09	1.4254 ± 0.0026
	Luteal phase	5	0.75 ± 0.10	1.4219 ± 0.0022
Pathology	Leimiosarcoma	2	0.72 ± 0.09	1.3597 ± 0.0031
	Stromal sarcoma	2	0.81 ± 0.07	1.4001 ± 0.0113
	Serous cancer	2	0.84 ± 0.08	1.4132 ± 0.0007
	Granulosa carcinoma	2	0.81 ± 0.10	1.4043 ± 0.0045

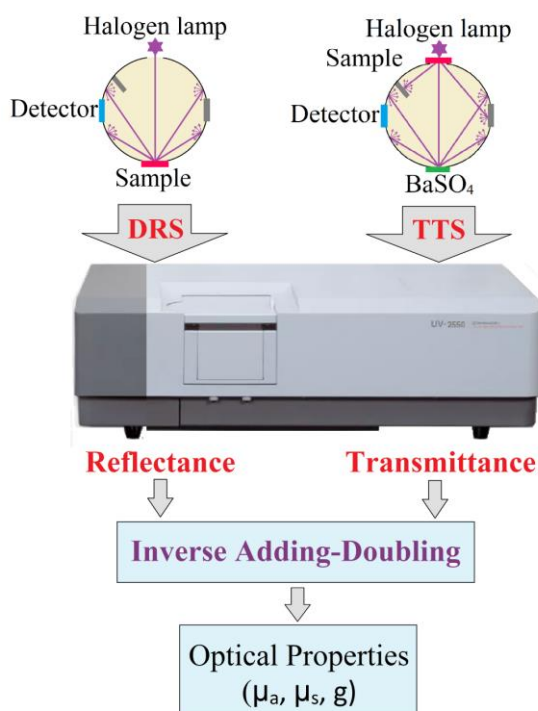


Fig. 1 Schematics of the experimental setup.

Each sample of the tissue under study was mechanically fixed in a special frame with a window of 0.5×0.5 cm and then in a quartz cuvette so that the tissue sample was pressed against the wall of the cuvette and subjected to optical measurement. The size of the inlet hole of the sphere is 2.2×1.2 cm. The sample was attached to the frame, and the free side was pressed tightly against the wall of the quartz cuvette without air gaps.

To measure total transmission spectra (TTS), a quartz cuvette containing a tissue sample was placed directly in front of the integrating sphere, collecting all radiation transmitted through the tissue sample. When measuring diffuse reflectance spectra (DRS), the cell with the sample was placed behind the integrating sphere, which collected all the radiation scattered by the sample (Fig. 1). The diameter of the light beam incident on the sample was 1 mm. Before measurements, a quartz cuvette with a fixed sample was filled with physiological solution to

moisten the sample and bring the measurement results closer to *ex vivo*.

The collimated transmission spectra of the samples were recorded using a setup that included a tungsten halogen radiation source LSH-4 (Biospek, Russia) and a QE-PRO polychromator (Ocean Optics, USA). The diameter of the collimated beam was 5.1 mm. Measurements were done with a blue filter and an acquisition time of 170 ms (Fig. 2). A blue filter was used to cut off the excess spectrum of the radiation source.

The refractive index was measured using a DR-M2/1550 multi-wavelength refractometer (Atago, Japan). An interference filter with a wavelength of 589 nm was used for measurements. Measurement accuracy was ± 0.0002 . During measurements using a circulation thermostat, the temperature was maintained at 22 °C. For calculations, we used the obtained refractive indices of tissue sections in normal conditions and with developed pathology (see Table 1). The obtained values correlate well with literature data [24].

The protocol of the Local Ethics Committee with permission to conduct these studies (no. 4, dated November 01, 2022) was issued by the Saratov State Medical University named after V. I. Razumovsky.

Based on the obtained values of collimated transmission (T_c), the attenuation coefficient was calculated:

$$\mu_t = -\frac{\ln[T_c]}{l}.$$

Then, because:

$$\mu_t = \mu_a + \mu_s,$$

knowing μ_t (from the collimated transmission spectra) and μ_a (obtained using IAD method), the scattering coefficient μ_s was calculated.

The IAD method allows one to determine the absorption coefficient μ_a and the transport scattering coefficient μ'_s of tissue using experimental data on the diffuse reflectance and total transmittance, calculations are described in detail in the work [25].

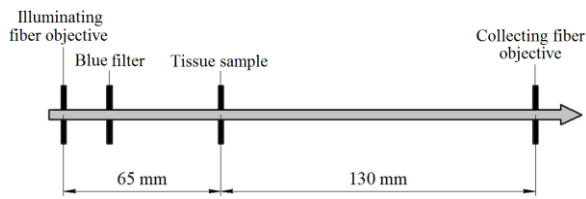


Fig. 2 Schematics of the collimated transmission measurement setup.

3 Results and Discussion

The optical properties of tissues (absorption coefficient μ_a , scattering coefficient μ_s and scattering anisotropy factor g) determine features of light propagation in tissues including its attenuation. Since the average refractive index of tissue is greater than that of air, at the

tissue–air interface part of the incident radiation is reflected (Fresnel reflection, about 4–7% of the incident intensity in dependence of the wavelength), and the rest penetrates into the tissue slab. As radiation passes through tissue, it undergoes scattering and absorption. When a photon is absorbed by a target molecule (endogenous chromophore), all its energy is transferred to this molecule, as a result of which it is converted into heat, re-emitted in the form of fluorescence and partially spent on biochemical reactions. If the light is not absorbed, then there is no effect on the tissue. In the UV region, the main endogenous chromophores of the studied samples are amino acid residues of proteins, collagen and elastin, and in the visible region the main chromophore is oxyhemoglobin, which has characteristic absorption bands at 415 nm (Soret band), 542 and 576 nm (Q-bands) (Fig. 3).

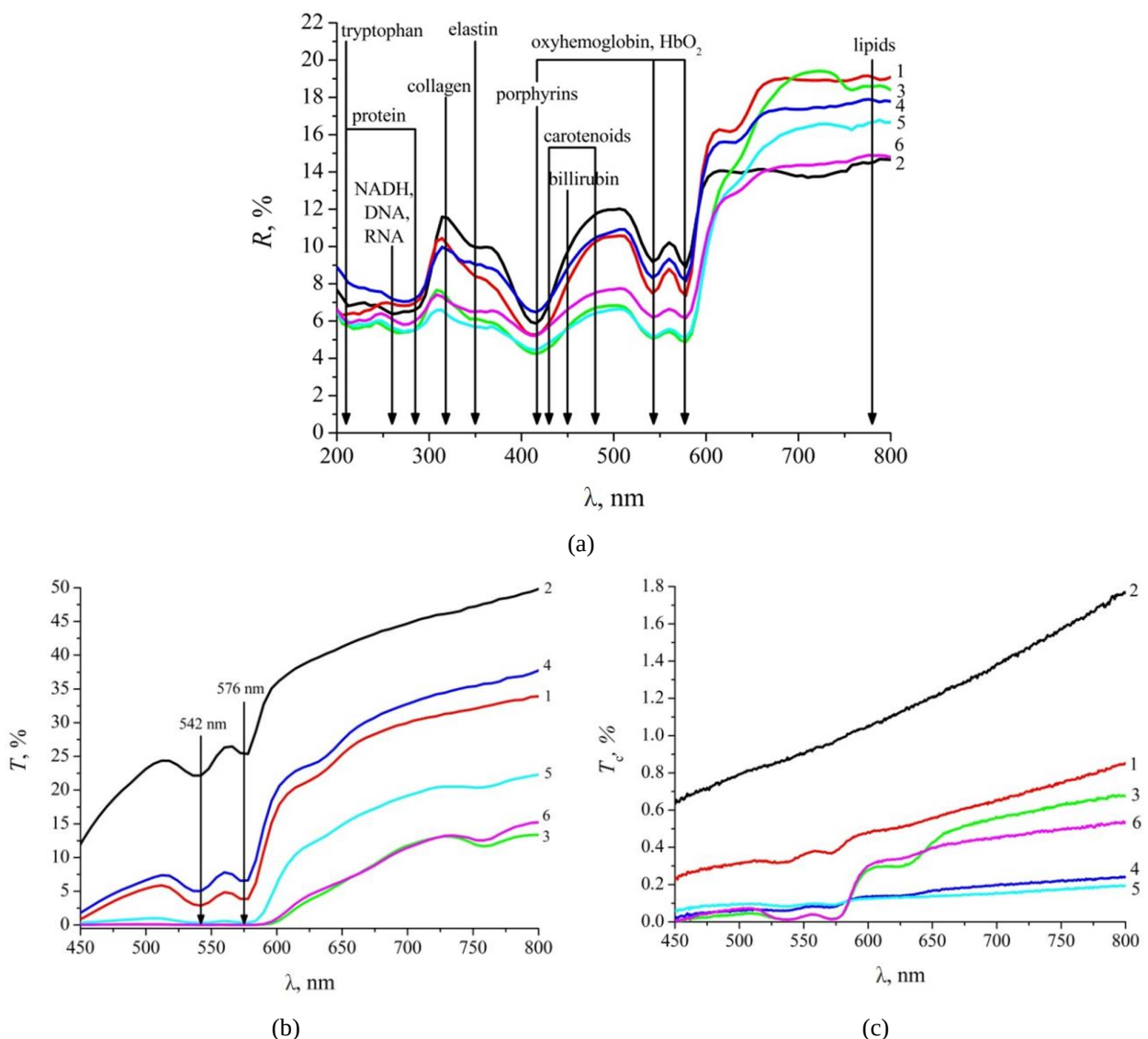


Fig. 3 DRS (a), TTS (b) and T_c (c) of the studied tissues sections of cat ovaries with indicated endogenous chromophores: 1 – leiomyosarcoma, 2 – stromal sarcoma, 3 – serous carcinoma, 4 – granulosa carcinoma, 5 – follicular phase (healthy), 6 – luteal phase (healthy).

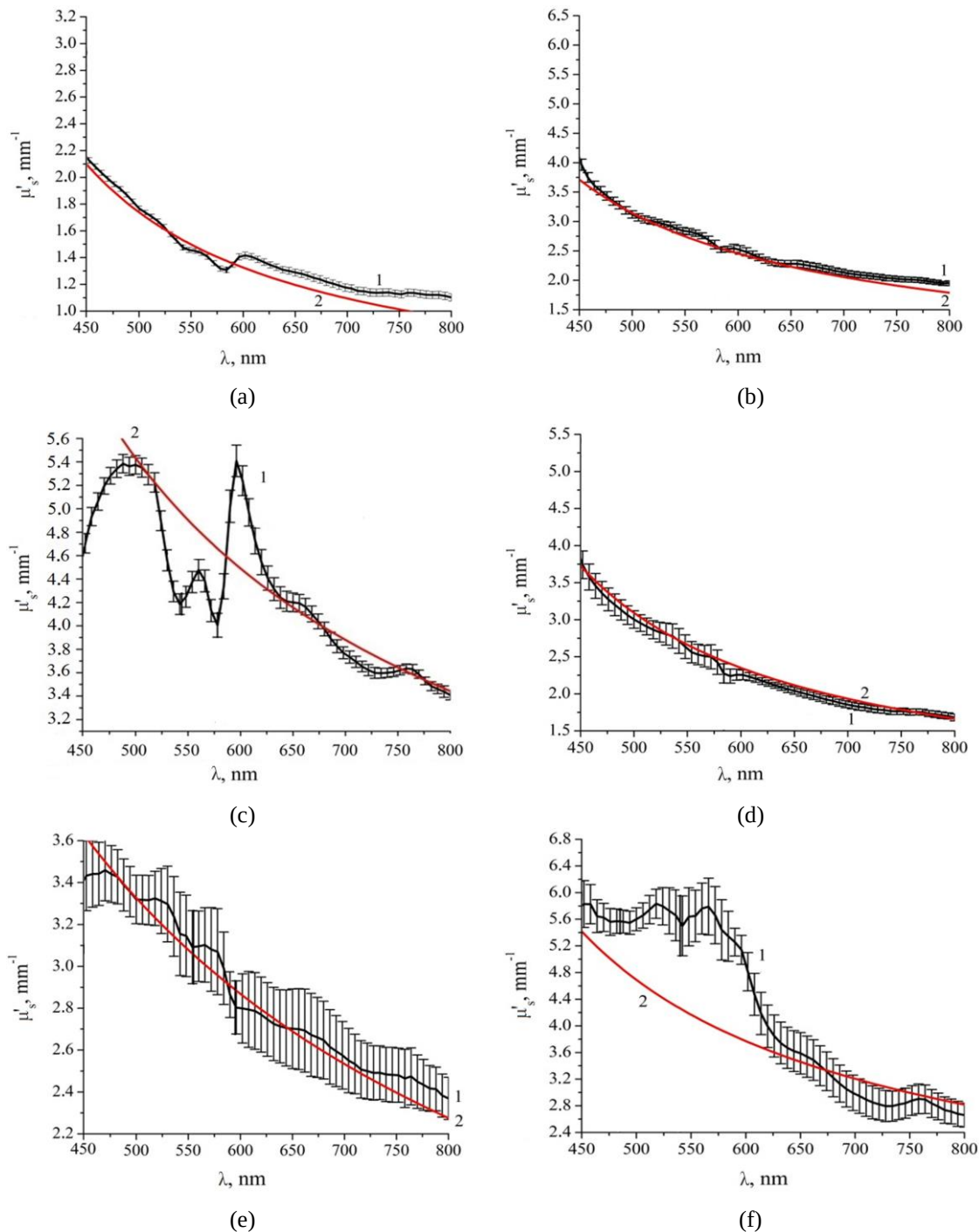


Fig. 4 Spectra of the transport scattering coefficient (1) and approximation graph (2) of the studied cat ovarian samples: (a) leiomyosarcoma (n=5); (b) stromal sarcoma (n=5); (c) serous carcinoma (n=5); (d) granulosa carcinoma (n=5); (e) follicular phase (healthy) (n=5); (f) luteal phase (healthy) (n=5). The standard deviation (SD) is shown by vertical lines.

For soft tissues, water and lipids are the most important endogenous chromophores, but in the studied optical range their absorption is negligible [26].

Another mechanism that attenuates radiation passing through tissues is scattering. This physical process directly depends on the size, shape and refractive index of cells, cellular organelles and various fibrous structures of tissues. The importance of the scattering phenomenon is that it rapidly reduces the energy flux density available

for absorption by the target chromophore in tissue depth. This determines the effectiveness of radiation exposure to tissue in clinical practice. Scattering decreases with increasing wavelength, forming at longer wavelengths between strong absorption bands of hemoglobin and water the so-called “diagnostic/therapeutic window of transparency” – an effective means of delivering energy to deeper tissue structures (600–1200 nm) [4]. In the TTS, all samples except stromal sarcoma have almost

zero transmission from UV up to 450 nm, and samples with serous carcinoma and luteal phase even up to 600 nm. For samples taken from ovaries in the follicular phase (normal), with leiomyosarcoma, granulosa carcinoma and leiomyosarcoma, the shape of DRS in the wavelength range from 400 to 800 nm correlates quite well with the shape of the transmission spectra of these

ovarian tissues (Fig. 3b). In this wavelength range the spectral shape is determined by strong absorption bands of oxyhemoglobin and the influence of light scattering by the main scatterers – collagen and elastin fibers. The DRS and TTS clearly show the absorption bands of oxyhemoglobin at wavelengths of 415, 542 and 576 nm.

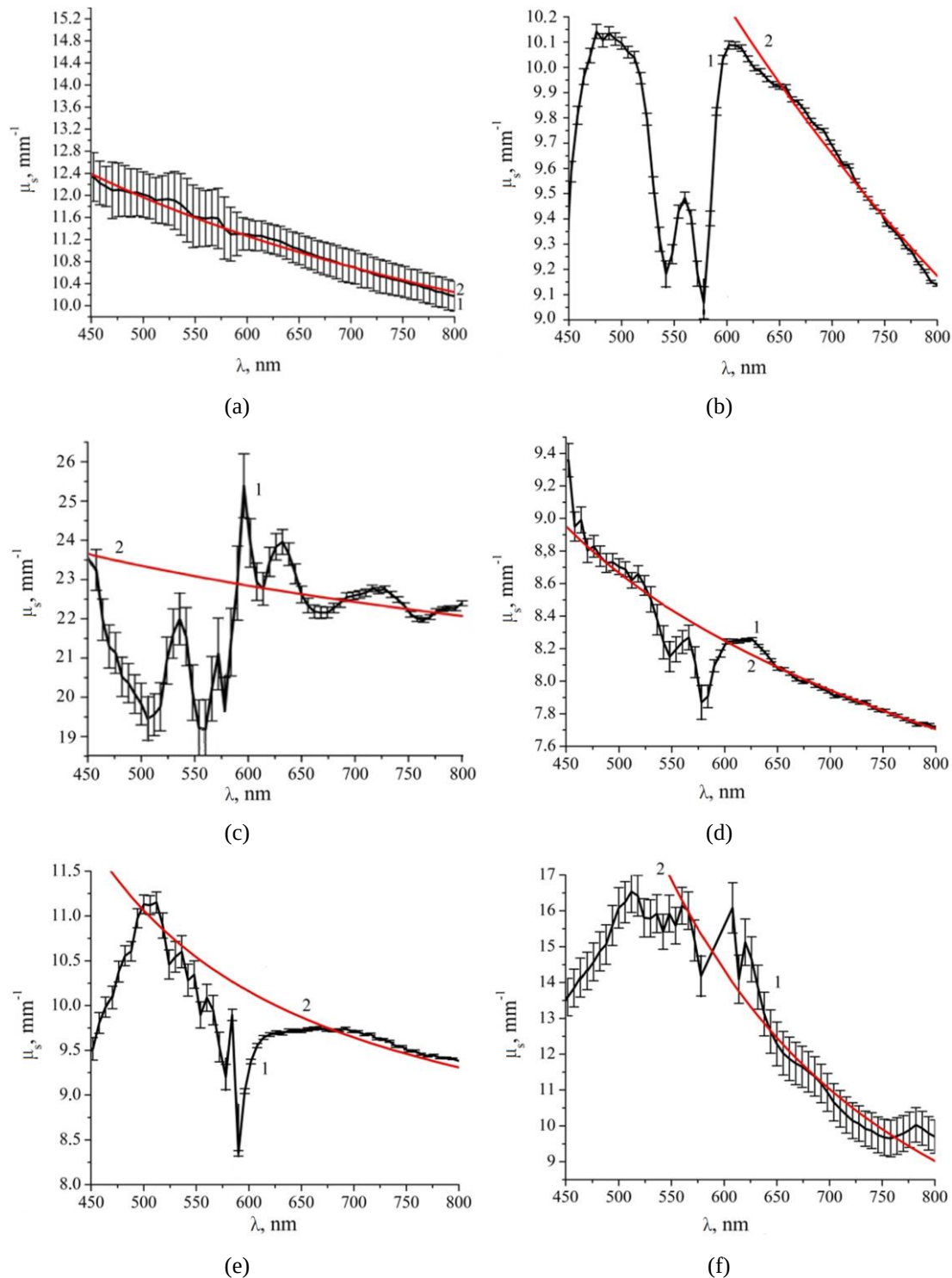


Fig. 5 Spectrum of the scattering coefficient (1) and approximation graph (2) of the ovaries of cats with diagnoses: (a) leiomyosarcoma (n=5); (b) stromal sarcoma (n=5); (c) serous carcinoma (n=5); (d) granulosa carcinoma (n=5); (e) follicular phase (healthy) (n=5); (f) luteal phase (healthy) (n=5). The standard deviation (SD) is shown by vertical lines.

Fig. 4 shows the transport scattering coefficient spectra calculated using the IAD method based on the measured of DRS and TTS. Taking into account the size of the probing beam incident on the surface of the tissue sample, the minimum sample size should be at least 9.5 mm, which is true for the smallest of the studied samples with dimensions of $12 \times 10 \text{ mm}^2$.

In Fig. 5 spectra of the transport scattering coefficient of the studied ovarian samples under normal conditions and with cancer pathology are presented, obtained by averaging the corresponding spectra measured for each of the 5 samples. The approximation was done using the least squares method. It can be seen that as wavelengths increase, the transport scattering coefficient smoothly decreases for all samples, which corresponds to the general nature of the spectral behavior of the scattering characteristics of many tissues. In samples diagnosed with serous carcinoma, the effect of deviation from the monotonic dependence is observed, which is explained by an increase in the influence of the oxyhemoglobin absorption.

According to Mie theory, the intensity of scattered radiation is determined by the complex refractive indices of scatterers and the surrounding medium [27]. An increase in the imaginary part of the complex refractive index in the region of strong absorption bands leads to a change in the scattering cross section and transport scattering coefficient, which is observed in the absorption region of one of the main endogenous chromophores of the samples, namely oxyhemoglobin at 415, 542 and 576 nm [28]. It was previously shown that in the region of the absorption bands of oxyhemoglobin with maxima 415, 542 and 576 nm, a decrease in g is observed, which inevitably leads to an increase in the transport scattering coefficient and the appearance of peaks in its spectrum at the corresponding wavelengths [29–31]. This effect can be observed in the transport scattering coefficient spectra of all studied samples of cat ovaries, both normal and oncological. The greatest effect, apparently caused by a high content of endogenous chromophore, is observed in serous carcinoma and in a healthy ovary in the luteal phase, which corresponds to literature data for tissues with a high blood content [32–35].

The ovary in the corpus luteum phase is the most blood-supplied organ of the whole organism due to an extensive network of anastomosis of blood vessels [36].

The least manifestation of peaks in the transport scattering coefficient spectra is observed in stromal sarcoma, which indicates the significant specificity of this pathology. The increase in the standard deviation observed in the region of absorption bands indicates a difference in the content of oxyhemoglobin for samples of tissues with different diagnoses. Shown in Fig. 4 data are in good agreement with the above. The presence of pronounced absorption bands leads to the fact that the formation of the spectrum occurs not only under the influence of the real, but also the imaginary part of the relative complex refractive index of the scattering centers of tissue, which manifests itself in the form of a local

decrease in the light scattering at the wavelengths, where absorption is strong. The monotonous spectral dependence of the transport scattering coefficient and the scattering coefficient is well approximated by a power function in the form $\mu'_s(\lambda), \mu_s(\lambda) \sim a\lambda^{-w}$, where parameter a is determined by the concentration of scattering centers of tissue and the ratio of the refractive indices of scatterers and their surrounding environment, and parameter w (wave exponent) characterizes the average size of scatterers and determines the spectral behavior of the scattering coefficient [21, 28, 37].

Fig. 4 shows the approximation of the spectra of the transport scattering coefficient, and Fig. 5 shows the approximation of the spectra of the scattering coefficient for ovaries with various diagnoses. The bars correspond to the standard deviation of the scattering characteristics obtained by averaging the measurements for five sections of each of the six types of ovaries (Table 1).

The scattering coefficient is characterized by the number of scattering events, which means that at wavelengths where absorption is strong, the number of scattering events may decrease, therefore dips are observed in the spectral dependence for the scattering coefficient. The transport scattering coefficient and absorption coefficient evaluated using IAD technique are strongly influenced by absorption bands of different forms of hemoglobin, in particular in studies of gingiva [20], colon tissues [18] and human gastric mucosa [37]. For our studies of cat ovaries besides hemoglobin absorption, absorption of carotenoids in the spectral range 420–480 nm can be significant [38]. The additional factor that affects deformation of the scattering coefficient spectral dependencies is anomalous dispersion in the vicinity of absorption bands, manifested by the wavelength shifts regarding the absorption bands [31, 33]. What can be observed in Figs. 4 and 5.

From Figs. 4 and 5 it is clear that the presented functions well approximate the experimental data in the spectral range of 500–800 nm. In the spectral region of 400–500 nm, a discrepancy is observed between the experimental data and the approximating dependences, which is associated with the influence of strong absorption of oxyhemoglobin at 415 nm (Soret band). Oxyhemoglobin also has two absorption peaks in the region under study, which appear in the spectra of DRS, TTS and T_c (Fig. 3) at wavelengths of 542 and 576 nm. They are visible in the spectra of the transport scattering coefficient (Fig. 4), scattering coefficient (Fig. 5), and the anisotropy factor (Fig. 6), and could be partly due to artifacts of the IAD algorithm. As expected, all three bands of oxyhemoglobin are clearly visible in the reconstructed absorption spectra (Fig. 7).

The absorption spectra can also be strongly influenced by various carotenoids that are found in human ovarian tissues under normal conditions and in oncological pathologies, namely beta-carotene, beta-cryptoxanthin, lutein, lutein epoxide, violaxanthin and mutatoxanthin at the same time antheraxanthin, hydroxyequinenone, and capsanthin are released sporadically [39].

Table 2 Approximation functions for the wavelength dependence of transport scattering coefficients $\mu'_s(\lambda)$ and scattering coefficient $\mu_s(\lambda)$ for the cat ovaries with various diagnoses, where λ is the wavelength (in nm).

Sample type	$\mu'_s(\lambda)$	$\mu_s(\lambda)$	$g(\lambda)$
Leimyosarcoma	$2.9 \cdot 10^{10} \cdot \lambda^{-4} + 183.9 \cdot \lambda^{-0.8}$	$2.15 \cdot 10^6 \cdot \lambda^{-8.1} + 111.9 \cdot \lambda^{-0.3}$	$0.79 + 0.1 \cdot [1 - \exp(-\frac{(\lambda-419.1)}{82.4})]$
Stromal sarcoma	$2.1 \cdot 10^{10} \cdot \lambda^{-3.9} + 352.2 \cdot \lambda^{-0.8}$	$2.15 \cdot 10^6 \cdot \lambda^{-5.6} + 103.1 \cdot \lambda^{-0.3}$	$0.74 + 0.07 \cdot [1 - \exp(-\frac{(\lambda-590.2)}{133.7})]$
Serous carcinoma	$3.0 \cdot 10^{10} \cdot \lambda^{-4} + 706.0 \cdot \lambda^{-0.8}$	$1.38 \cdot 10^6 \cdot \lambda^{-5.4} + 49.2 \cdot \lambda^{-0.1}$	$0.76 + 0.1 \cdot [1 - \exp(-\frac{(\lambda-520.7)}{149.4})]$
Granulosa carcinoma	$1.5 \cdot 10^{10} \cdot \lambda^{-3.8} + 319.5 \cdot \lambda^{-0.8}$	$1.02 \cdot 10^8 \cdot \lambda^{-3.4} + 18.2 \cdot \lambda^{-0.1}$	$0.61 + 0.21 \cdot [1 - \exp(-\frac{(\lambda-459.3)}{177.6})]$
Follicular phase (healthy)	$1.8 \cdot 10^9 \cdot \lambda^{-4.1} + 477.7 \cdot \lambda^{-0.8}$	$1.01 \cdot 10^8 \cdot \lambda^{-3.3} + 21.4 \cdot \lambda^{-0.1}$	$0.66 + 0.11 \cdot [1 - \exp(-\frac{(\lambda-469.4)}{217.9})]$
Luteal phase (healthy)	$1.4 \cdot 10^{10} \cdot \lambda^{-3.8} + 564.7 \cdot \lambda^{-0.8}$	$5.02 \cdot 10^8 \cdot \lambda^{-2.8} + 93.7 \cdot \lambda^{-0.4}$	$0.74 + 0.11 \cdot [1 - \exp(-\frac{(\lambda-880.6)}{534.9})]$

These carotenoids have typical spectra with strong absorption bands at wavelengths (413–426 nm), (437–453 nm) and (465.5–480 nm) [40]. For example, the bands of beta-carotene are centered at 426, 453 and 480 nm, and those of lutein – at 423, 446 and 474 nm. The overlapping combination of these bands may be responsible for the strong rise in absorption spectra in the range from 440 to 480 nm for tissues in the follicular phase, luteal phase and serous cancer. A peak at a wavelength near 446 nm is clearly visible in the spectra of the tissue sample in the luteal phase.

The obtained approximation functions for the wavelength dependence of transport scattering coefficients and scattering coefficient are presented in Table 2.

In total 84 samples (tissue sections) were examined. To clarify diagnoses, 18 sections (3 from each of 6 ovary types) were used for histological studies. To measure the refractive index 18 sections (3 from each of 6 ovary types) and for spectral measurements (see Table 1), 48 sections (5 and 3 from each of 6 ovary types for DRS/TTS measurements and collimated transmission, respectively) were used.

The fact that the approximating functions for both the transport scattering coefficient and the scattering coefficient are a combination of two power functions indicates that the spectrum of the transport scattering coefficient is formed by at least two types of scatterers. The first term of the approximating function is responsible for light scattering caused by sufficiently small scatterers (Rayleigh scattering), as which may appear, for example, as individual collagen fibers, etc. The second term corresponds to fairly large scatterers, the so-called Mie scatterers, which can be fiber bundles or their plexuses, as well as cell nuclei, mitochondria, follicles, corpus luteum structures, fibrous scar tissue or other fairly large components.

It is clearly seen that in the region of 450–800 nm the scattering coefficient smoothly decreases towards longer wavelengths, which generally corresponds to the general nature of the spectral behavior of the scattering characteristics of biological tissues. However, in the

range of 410–600 nm, peaks appear in the region of the absorption bands of hemoglobin and its oxygenated forms, which is explained by the increase in the influence of the imaginary part of the complex refractive index of scattering centers in this region [16–20].

The formation of the transport scattering coefficient and the scattering coefficient for all studied samples occurs by at least two types of scatterers, since the approximation functions are a combination of two power functions. The first term of the approximating functions is responsible for light scattering caused by fairly small scatterers, which can be nanovesicles 20–150 nm in size, identified in the ovaries [41], individual collagen fibers, etc. The second term corresponds to fairly large scatterers (Mie scatterers), which can be, in particular, lipid granules. Recent studies have shown that lipids in mammalian ovaries are not only evenly distributed, but also occur in the form of cytoplasmic lipid granules. Lipid granules in oocytes and embryos are not only repositories of energy substrates, but also active intracellular structures that perform many other functions [42]. For most mammalian cells, the diameter of lipid granules typically varies in the range of 0.1–1.0 μm [43].

The hydrophobic core of lipid granules, surrounded by a phospholipid monolayer, is mainly composed of triacylglycerols and sterol esters such as cholesterol [44]. Lipid granules also contain various proteins [45].

Species with oocytes in the ovaries and lipid-rich embryos include pig, rat, sheep, cat and dog [46]. Lipids have a refractive index of ≈ 1.45 , and water 1.33 [3, 4]. The discrepancy between the refractive indices of lipid granules and the cytoplasm leads to intense light scattering on lipid droplets. The measured refractive indices of healthy cat ovaries in the follicular and luteal phases were 1.4254 ± 0.0026 and 1.4219 ± 0.0022 , respectively. With the oncological diseases under consideration, the refractive index of ovarian tissue decreases and is: serous cancer 1.4132 ± 0.0007 ; granulosa carcinoma 1.4043 ± 0.0045 ; stromal sarcoma 1.4001 ± 0.0113 ; leimiosarcoma 1.3597 ± 0.0031 , which suggests a decrease in lipids in their composition.

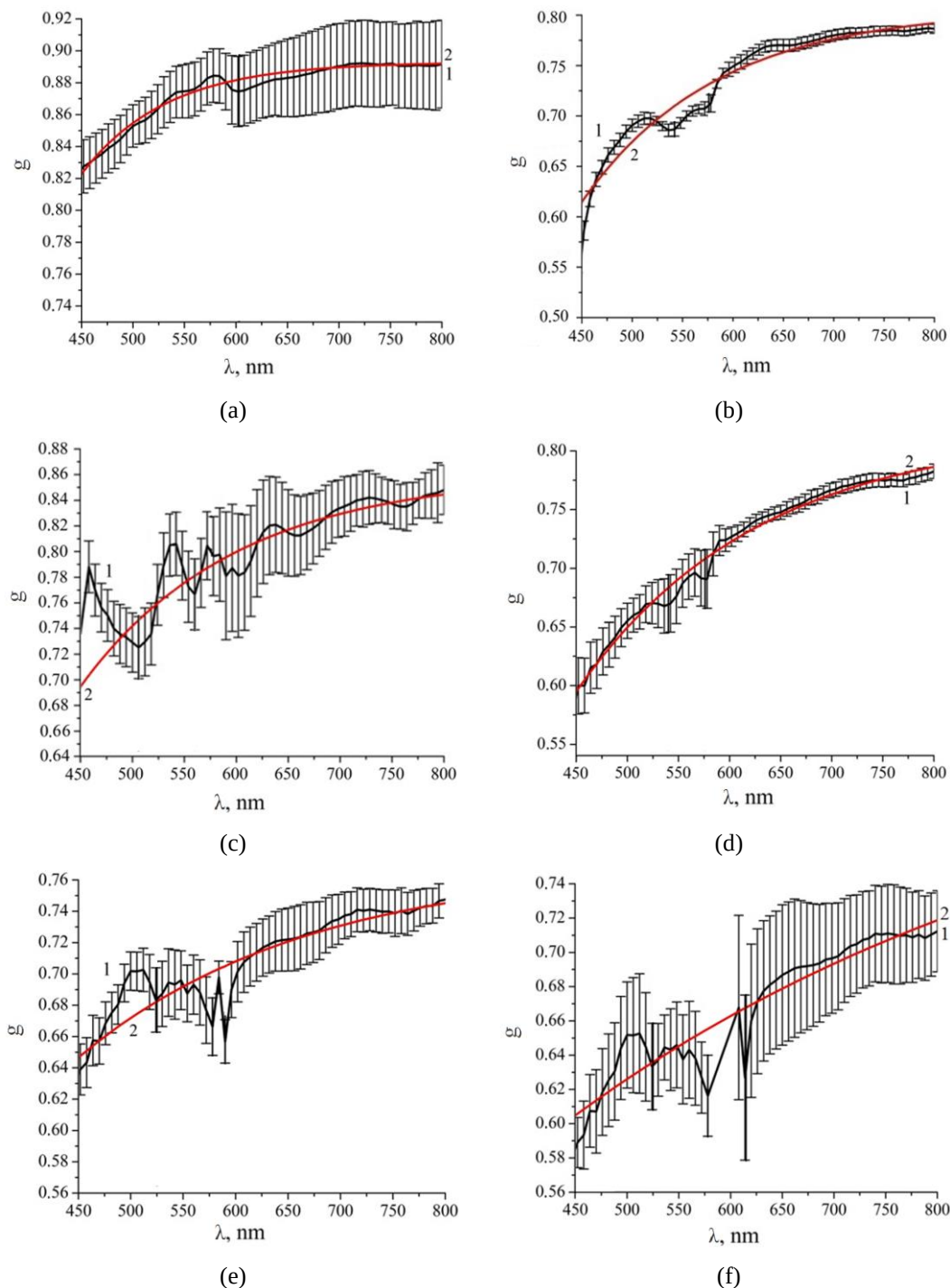


Fig. 6 Spectral dependences of scattering anisotropy factors (1) and approximation graphs (2) of the studied samples of cat ovaries with various diagnoses: (a) leimiosarcoma ($n=5$); (b) stromal sarcoma ($n=5$); (c) serous carcinoma ($n=5$); (d) granulosa carcinoma ($n=5$); (e) follicular phase (healthy) ($n=5$); (f) luteal phase (healthy) ($n=5$). The standard deviation (SD) is shown by vertical lines.

Also, large scatterers are follicles, which are vesicles, the wall of which is formed by epithelial cells, thyrocytes, and colloid inside. The size of the follicles has a diameter depending on the phase of maturation from 400 to 2000 μm [47].

In the luteal phase, a significant part of the ovary is occupied by the corpus luteum – this is a temporary endocrine gland, containing small luteal cells

($12 \pm 3 \mu\text{m}$) and large luteal cells ($34 \pm 5 \mu\text{m}$), as well as a significant number of lipid droplets [48].

In leimyosarcoma, pronounced autolytic fragments of the tumor identified during histological examination can be significant scatterers, and atypical spindle-shaped cells can be small.

Histological examination in the ovaries with serous carcinoma revealed numerous cystic formations, subepithelial formations, large-focal numerous

hemorrhages, foci of infiltration by siderophages; tubular, pseudotubular and solid structures formed by large hyperchromatic tumor cells can act as both partially large and small scatterers.

With stromal sarcoma, the identified focal moderate polymorphocellular subacute infiltrate, represented by lymphocytes, plasma cells, macrophages, neutrophils short intertwined bundles, turning into continuous fields of underdeveloped connective tissue, can serve as large scatterers.

During histological examination, fragments of fibrovascular tissue with solid and focally cystic tumor structures, predominant mucytous fields, zones of necrosis and acute inflammatory infiltration identified in samples with granulosa cell carcinoma and scattered polymorphous cells with eosinophilic cytoplasm and rounded nuclei can also act as quite large scatterers.

The pathologies of stromal sarcoma, leiomyosarcoma and granulosa cell carcinoma contain an increase in collagenous stromal structures identified histologically, which act as large scatterers with the lowest absorption coefficients in the visible range (Fig. 7). The experiments carried out by measuring changes in the mass of samples in air made it possible to determine the amount of interstitial water as a percentage of the initial mass, which was: for the luteal phase 71.1%; for the follicular phase 72.1%; for leiomyosarcoma 47.9%; for serous carcinoma 76.8; for granulosa cell carcinoma 46.5; for stromal sarcoma 51.4%. Thus, the pathology of serous carcinoma contains a larger amount of interstitial water with hemoglobin in oxygenated form relative to the norm, which is evident in the almost doubling of scattering (Fig. 5c) and significant values of absorption coefficients (Fig. 7).

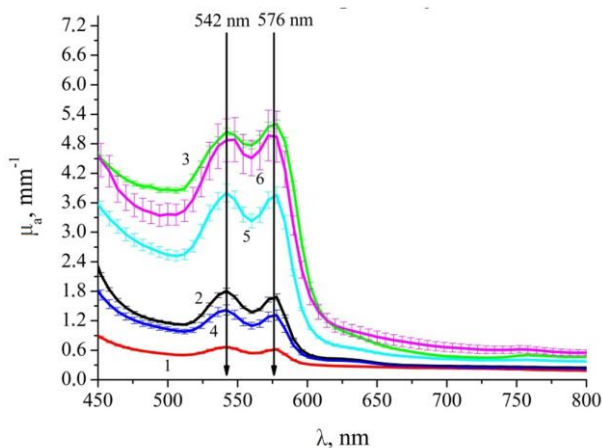


Fig. 7 Absorption spectra reconstructed from DRS and TTS measurements for all studied ovarian samples with various diagnoses: 1 – leiomyosarcoma ($n=5$); 2 – stromal sarcoma ($n=5$); 3 – serous carcinoma ($n=5$); 4 – granulosa cell carcinoma ($n=5$); 5 – follicular phase (healthy) ($n=5$); 6 – luteal phase (healthy) ($n=5$). The standard deviation (SD) is shown by vertical lines.

It is clearly seen that in the region of 450–800 nm the scattering coefficient smoothly decreases towards longer

wavelengths, which corresponds to the general nature of the spectral behavior of the scattering characteristics of biological tissues [16–20]. However, in the range of 410–600 nm, peaks appear in the region of the absorption bands of hemoglobin in its oxygenated form, which is explained by the increase in the influence of the imaginary part of the complex refractive index of scattering centers in this region.

Fig. 6 shows the spectral dependences of the scattering anisotropy factor of ovaries in norm and with various diagnoses. It is clear seen that the experimental data for $g(\lambda)$ are fairly well approximated by the functions, presented in Table 2.

The spectral dependences of the scattering anisotropy factor increase with increasing wavelength and have a form characteristic for other biological tissues [16–20]. In the visible region, its formation also occurs under the influence of both small and large particles, while in the IR region the main contribution is made only by fairly large scatterers, as evidenced by the increase in g with increasing λ . Sharp dips in the region of absorption bands of hemoglobin and its oxygenated form are explained by the influence of the imaginary part of the complex refractive index of both the scatterers themselves and their environment.

Most biological tissues exhibit anisotropic properties, which are primarily manifested in scattering anisotropy and are quantitatively characterized by the scattering anisotropy parameter. The factor for various tissues ranges from 0.7 to 0.93 [3, 4], as can be seen from the obtained spectral dependences for the samples under study. An increase in the anisotropy coefficient with increasing wavelength is also typical for most biological tissues [17, 28].

In the absorption spectra reconstructed from DRS and TTS measurements for all studied ovarian samples (see Table 1) with various diagnoses presented in Fig. 7, absorption bands of oxyhemoglobin are visible with maxima at 415, 542 and 576 nm. The presence of strong absorption bands reduces the number of both transmitted and backscattered photons for the wavelengths within the absorption bands. Starting from 650 nm and further up to 800 nm, the influence of hemoglobin absorption bands ceases to be significant; the total transmittance and back reflectance spectra are formed mainly due to scattering, since the influence of the absorption bands of tissue chromophores in this region is minimal, which corresponds to their transparency window. The spectra show that the bands do not shifted and appear quite intensive for all sample types. However, absorption is minimal in leiomyosarcoma, in granulosa cell carcinoma and stromal sarcoma it is also rather small, which is explained by the lower blood supply and small containing of carotenoids in the ovaries with these diagnoses. Despite the fact that granulosa cell carcinoma in the ovary has multiple cavities filled with blood-containing fluid, the absorption coefficient at the oxyhemoglobin wavelength of 576 nm does not exceed 1.3 mm^{-1} . The greatest blood supply is in samples diagnosed with the luteal phase of a healthy ovary and serous carcinoma, the

absorption coefficient at 576 nm reaches 5.2 mm^{-1} . Also, tissues in both phases of a healthy ovary and serous carcinoma apparently contain different types of carotenoids, which is clearly visible from the rise in the absorption spectrum at wavelengths of 440–500 nm.

The results of measurements show that there are significant differences in the ratio of scattering to absorption coefficient (μ_s/μ_a) between normal and cancer tissues, except for serous carcinoma, which is a superficial lesion of the organ and manifests itself in the epithelial tissue layer. The (μ_s/μ_a) ratio calculated at 500 nm is the highest for leiomyosarcoma (24), moderate for stromal sarcoma (8.5) and granulosa carcinoma (7.3), and the smallest for serous carcinoma (4.6), luteal phase (healthy) (4.6) and follicular phase (healthy) (4.4). The wavelength of 500 nm is between the strong hemoglobin absorption bands, where light scattering and absorption of hemoglobin and carotenoids are still significant.

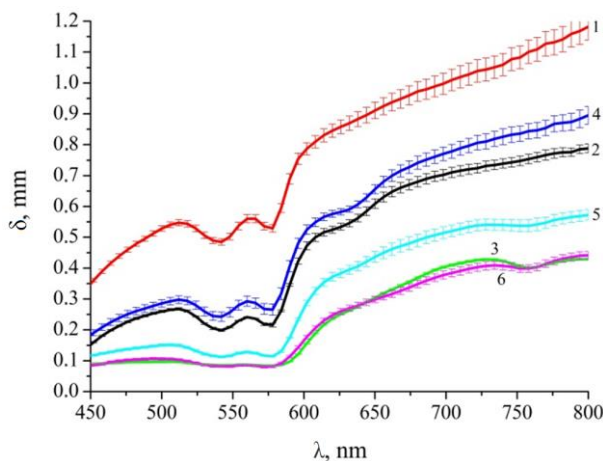


Fig. 8 Dependence of the light penetration depth (δ) into the tissue of the studied ovarian samples with various diagnoses on the wavelength, calculated from the experimental data presented in Figs. 4 and 7: 1 – leiomyosarcoma ($n=5$); 2 – stromal sarcoma ($n=5$); 3 – serous carcinoma ($n=5$); 4 – granulosa carcinoma ($n=5$); 5 – follicular phase (healthy) ($n=5$); 6 – luteal phase (healthy) ($n=5$). The standard deviation (SD) is shown by vertical lines.

For organs heavily saturated by blood, the tissues to be tested can be pre-washed by perfusion with isotonic saline in order to avoid strong influence of blood absorption on the measured scattering properties of tissues. As it done recently in studies of sections of rat liver tissue, to reduce the effect of blood absorption on the recorded scattering properties [49].

From a comparison of the transport scattering coefficient of the studied samples (Fig. 4), it is clear that despite the fact that in general the spectral behavior of the transport scattering coefficient for all types of tissues is similar, light scattering for leiomyosarcoma more than 3 times less (at 500 nm) than for samples with serous carcinoma, which has the most abnormal behavior due to

the pathology characteristic of this type of tumor. Ovarian tissue in the luteal phase has a significant scattering up to 600 nm. Strong light scattering is also observed in ovaries diagnosed with stromal sarcoma and granulosa cell carcinoma. A healthy ovary in the follicular phase of the cycle has medium light scattering properties among all studied ovaries. The obtained data correlate well with literature data [11, 12]. In these papers, optical coherence tomography (OCT) with the wavelength 1300 nm was used to distinguish healthy from cancerous ovarian tissue (without specifying the specific tumor type) by estimation attenuation coefficient for ballistic photons which is very close to tissue scattering coefficient. The normal group has a higher scattering coefficient at 1310 nm ranging from 0.60 to 5.27 mm^{-1} with a mean value of $2.38 \text{ mm}^{-1} (\pm 0.67)$, while the malignant group shows a lower value in the range from 0.42 to 3.86 mm^{-1} with a mean value of $1.74 \text{ mm}^{-1} (\pm 0.55)$. The average scattering coefficient obtained from the normal tissue group consisted of 833 measurements from 88 sites was $2.41 \text{ mm}^{-1} (\pm 0.59)$, while the average coefficient obtained from the malignant tissue group consisted of 264 measurements from 20 sites was $1.55 \text{ mm}^{-1} (\pm 0.46)$ [11]. This difference may be related to the collagen content in healthy and cancer tissues [12].

For clinical applications of phototherapy and laser surgery, as well as optical diagnostics, it is important to know the depth of light penetration into tissue. Using Eq. $\delta = 1/\sqrt{3\mu_a(\mu_a + \mu'_s)}$, we determined the depth of radiation penetration into the cat ovarian tissue (Fig. 8) [17]. It can be seen that depending on the wavelength of the probing radiation, the depth of its penetration changes significantly. The greatest depth of penetration was found in samples with leiomyosarcoma. Granulosa cell carcinoma and stromal sarcoma have medium values. The lowest values of radiation penetration depth were found in samples diagnosed as healthy ovaries in the luteal and follicular phases and serous cancer, which may be due to the highest content of blood and carotenoids in these samples.

4 Conclusion

In this work, the optical properties of the ovarian tissues of cats with the diagnoses leiomyosarcoma, stromal sarcoma, serous carcinoma, granulosa carcinoma, follicular phase (healthy), and luteal phase (healthy) were experimentally studied in the wavelength range from 400 to 800 nm. Based on the measured spectra of diffuse reflectance, total transmittance and collimated transmittance spectra, spectral dependences for absorption and reduced scattering coefficients were determined using the IAD method, as well as for scattering anisotropy factor and the light penetration depth into ovarian tissue in normal conditions and in various types of cancer. The results of measurements demonstrated that there are significant differences in the ratio of scattering to absorption coefficient μ_s/μ_a at 500 nm between normal and cancer tissues, except for

serous carcinoma, which is a superficial lesion of the organ and manifests itself in the epithelial tissue layer. We were able to reveal that scattering predominates over absorption in all types of tumors under consideration (except for serous carcinoma), which indicates large differences in the composition and structure of the studied samples in normal and malignant conditions, which is possibly due to the high collagen content in cancer samples. These observations are consistent with literature data for other tissues. The difference in the optical properties of healthy and malignant ovaries can serve as an important and promising marker for monitoring of pathology development in the early stages in clinics using optical methods.

Disclosures

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author Contributions

Authors are equally contributed to this work.

Acknowledgments

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Effect of Upconversion Nanoparticles on Erythrocytes

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Abstract. We have shown experimentally that the interaction of upconversion nanoparticles with erythrocytes leads to changes in cell size and shape. One of the reasons for this is thought to be a change in the viscosity of erythrocyte membrane. Using a fluorescent probe, pyrene, we have shown that this nanoparticle-induced change in membrane viscosity depends on the nature of the nanoparticle shell (cover). © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: erythrocytes; nanoparticles; morphology; membrane viscosity.

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1 Introduction

The widespread prevalence of oncological diseases requires the search for and development of various treatment options. Chemotherapy, radiotherapy, surgical treatment, and photodynamic therapy are among the existing ones [1]. Photodynamic therapy is based on the generation of reactive oxygen species using visible or near infrared (IR) radiation in combination with a photosensitizer [2].

In photodynamic therapy, the main problem is the delivery of the photosensitizer to the area to be treated. There is also a need for direct measurement of thermal fields within the tissue in the immediate vicinity of the treatment area, as intensive radiant heating of the tissue can cause negative side effects.

One way of solving this problem is to use of nanoparticles, which can act as both photosensitizer carriers and nanothermometers at the same time. The use of nanoparticles in medicine plays an important role in the development of drugs and their delivery routes. One of the main advantages of nanoparticles is their ability to deliver drugs directly to the desired cells and organs of the body. Because of their size, nanoparticles can penetrate biological barriers such as cell membranes, allowing targeted drug delivery. In addition, nanoparticles can be designed so that their luminescence parameters depend on the ambient temperature, with the most promising nanothermometers being ZnCdS [3], CuInS₂ [4], and NaYF₄Er,Yb [5, 6].

Today's technologies allow the synthesis of nanoparticles of various sizes from ~ 5 to 100 nm raising the question of their safe application in medicine. The application of anticancer drugs usually starts with an injection into the bloodstream to be transported to the tumour site. Such nanoparticles can then penetrate and accumulate in various organs: brain, liver, kidney, lung, and spleen [7].

There are a number of requirements for nanoparticles used in medicine, including biocompatibility, safety of use, and low toxicity being among them.

When introduced into the bloodstream, nanoparticles can attach to or penetrate the membrane blood cells. The most common blood cells are red blood cells. They represent a natural carrier for various bioactive substances and contrast agents due to their unique properties such as biocompatibility, membrane flexibility [8].

Nanoparticles have previously been shown to interact with erythrocytes and alter cell structure and function [3, 4]. Nanoparticles interacting with erythrocyte membranes alter the microviscosity and mechanical stresses of the lipid bilayer [3, 4]. As a result, the changes that occur in the membranes affect some functions of erythrocytes, such as the ability of cells to pass through microcapillaries [9].

Therefore, when planning a therapeutic application of nanoparticles, it is necessary to be sure that the interaction of nanoparticles with the erythrocyte

membrane does not lead to fatal morphological and physiological consequences.

The aim of this work is to observe the change of erythrocyte membrane properties under the action of NaYF₄ nanoparticles with different surface conditions.

2 Materials and Methods

The synthesis of nanoparticles was carried out according to the following protocol.

Solutions 1 M of Y(NO₃)₃ (3 mL), Yb(NO₃)₃ (0.6 mL), and Er(NO₃)₃ (0.04 mL) were mixed. Then solutions of sodium citrate (Na₃C₆H₅O₇, 1 M, 4 mL) and citric acid (C₆H₈O₇, 3.66M, 50 mL) were added successively. At the resulting concentration of citrate groups, the rare earth (RE) citrate complexes are water soluble, so the solution was stirred until completely clear. Then 38 mL of 2 M sodium fluoride (NaF) solution was added and the resulting gel-like solution was stirred for 30 min.

As a result, the following molar ratios were obtained: Y:Yb:Er = 1:0.2:0.013, RE: fluorine = 1:19, RE:citrate = 1:51.4. Near stoichiometry was achieved for sodium.

The solution was poured into a 100 mL reactor, hermetically sealed, and heated at 180 °C for 18 h. After synthesis, the reactor was allowed to cool naturally. The synthesized nanoparticles were washed three times with distilled water with intermediate precipitation by centrifugation (centrifugation: Eppendorf 5804 (Eppendorf Germany), operating mode 6000 rpm = 4200 rcf, 15 min, T = 22 °C) and then air dried. To partially replace the citrate ions with BF⁻⁴, the nanoparticles were mixed with a solution of NaBF₄ in dimethylformamide at a ratio of 60 mg NaBF₄ to 25 mg of nanoparticles. The mixture was stirred for 20 h using a PTR-25 mini-rotator (Russia). The nanoparticles were then washed three times with ethanol and dried.

To coat the surface of the nanoparticles with SiO₂ shell, 80 mg of synthesised NaYF₄:Er,Yb particles were added to 1 mL of isopropyl alcohol and treated with ultrasound. Distilled water (7 mL), 13% ammonia solution NH₃(aq.) (1 mL), suspension of NaYF₄:Er,Yb particles in alcohol (1 mL), and tetraethoxysilane (TEOS) (80 µL) were added to of isopropyl alcohol (31 mL). The resulting mixture was stirred for 2.5 h. The white precipitate was then separated by centrifugation, washed three times in water, and dried at 70 °C for 20 h.

To coat the nanoparticles with an albumin shell, 1 mL of 2 mg/mL solution of commercial human serum albumin (HSA) (Sigma-Aldrich, USA) conjugated with the photodynamic dye Cy₃ was added to 10 mL of nanoparticle suspension (40 mg) in 0.05 M phosphate buffer at pH 6.5. The suspension was incubated for 0.5 h with vigorous stirring using a magnetic stirrer. Then 30 µL of 3% hydrogen peroxide solution was added and stirred for a further 0.5 h. The nanoparticles were precipitated by centrifugation (centrifugation: Eppendorf 5804 (Eppendorf, Germany), operating mode 6000 rpm = 4200 rcf, 10 min, T = 22 °C). The precipitate was resuspended in washing buffer and then precipitated

again. The procedure was repeated three times, after which the nanoparticles were suspended in 10 mL of buffer solution.

The attachment of folic acid to the albumin-coated nanoparticles was carried out by standard EDC/NHS covalent binding. For this purpose, 40 mg of folic acid (C₁₉H₁₉N₇O₆), 14 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 10.4 mg of N-hydroxysuccinimide (NHS) were dissolved in 2.8 mL of dimethyl sulfoxide (DMSO). The solution was stirred for 30 min and then added dropwise to the nanoparticle suspension under vigorous stirring. The resulting mixture was then stirred for 3 h at room temperature. The nanoparticles were precipitated by centrifugation, washed three times with saline solution, and then resuspended in this solution at the concentration of 4 mg/mL. The resulting nanoparticle suspension, which acquired a yellow colour characteristic of folic acid was stored in the dark at 4–6 °C. The presence of folic acid on the surface of the nanoparticles was monitored by IR spectroscopy.

The interaction of nanoparticles with erythrocyte membranes was studied in white Wistar rats. All the work with experimental animals was performed in accordance with the research protocol, which does not contradict the Geneva Convention of 1985 on “International Principles of Biomedical Research Using Animals” and the Helsinki Declaration of 2000 on the humane treatment of animals, as well as in accordance with the provisions of the Order № 755 of the Ministry of Health of the USSR dated August 12, 1977. The experiments were carried out on the basis of the Collective Use Centre (CUC) of the Research Institute of Basic and Clinical Urology of V. I. Razumovsky Saratov State Medical University of the Ministry of Health of the Russian Federation.

Experiments were performed in rats with liver cancer and healthy controls. White laboratory rats, weighing 300 ± 50 g, were implanted subcutaneously in the region of the scapula with 0.5 ml of a 25% tumour (alveolar liver cancer strain PC-1) suspension in Hanks' solution. The experiment was started when the tumours reached a volume of 1 cm³. A suspension of NaYF₄:Er,Yb nanoparticles with different shells (coated 1) SiO₂, 2) human serum albumin (HSA), 3) HSA and folic acid, 4) HSA-folic acid-photodynamic dye Cy₃ complex) was injected intravenously, and blood samples were taken one day after nanoparticle injection.

As a control, blood was taken from healthy and cancerous rats that had not been injected with nanoparticles.

Heparin-stabilized rat blood samples obtained from experimental or control animals were diluted with twice the volume of physiological solution and washed in a centrifuge for 5 min at 3000 rpm (centrifugation: ОПН-8УХЖ14.2 (Dastan, Kyrgyzstan), operating mode 3000 rpm = 930 rcf, T = 22 °C). After centrifugation, 0.02 ml of erythrocyte mass was removed from the bottom of the tube and added to a tube containing 1.8 ml of saline. Then, 0.5 ml of physiological solution was added to 0.5 ml of the previously obtained erythrocyte

suspension to obtain the working concentration of erythrocytes.

Observation of the changes in shape and size of erythrocytes in cancer control and after interaction with the nanoparticle suspensions was performed on the erythrocyte suspensions described above using a LOMO Mikmed-2 microscope. This microscope allows qualitative and quantitative assessment of the shape and internal structure of transparent biological microobjects. A Videoscan-415/P-USB camera with a 776×582 matrix was used to obtain the micrographs.

The recorded images were processed using the ImageJ software.

The following indices were used to characterize the size and shape of blood cells.

1) Area – the area of the horizontal projection of the erythrocyte,

2) Feret – maximum Feret diameter. It corresponds to the smallest diameter of the circle circumscribed around the object, *i.e.* the greatest length of the object. Instrumentally, this length is obtained by measuring the length with a caliper, and, *e.g.*, in pomology, for example, it is called the largest linear diameter, the longitudinal diameter. In cell microscopy, it is the diameter of the circle circumscribed around a cell of any shape.

3) MinFeret – minimal Feret diameter. It corresponds to the smaller diameter of the circle inscribed in the object. It can be obtained by measuring the transverse diameter of the object with a caliper.

We proposed to introduce a coefficient equal to the ratio of the diameters of the inscribed and circumscribed circles $k = \text{MinFeret}/\text{Feret}$. This coefficient indicates the degree of shape change of erythrocytes. When the shape of the erythrocytes changes to a stellate shape, *i.e.* the echinocytes are formed, the size of the circumscribed circle increases, while the diameter of the inscribed circle decreases, in which case the ratio of the diameters characterizes the degree of shape change.

In order to analyse the reasons for changes in erythrocyte shape, experiments were proposed to assess the structural and dynamic state of cell membranes using a fluorescent probe.

To analyse the microviscosity of erythrocyte membranes, we used a fluorescent probe, pyrene. In membranes, pyrene can be incorporated into the lipid bilayer. Upon photoexcitation, pyrene monomers fluoresce in the range of 370–400 nm. Also upon photoexcitation, pyrene monomers are able to form excimers with emission maximum at 470 nm. The ratio of the fluorescence intensity of excimers and pyrene monomers, *i.e.* the pyrene excimerization coefficient (F_e/F_m), depends on the lateral diffusion rate of the probe in the bilipid layer. The higher is the diffusion rate of the probe, the lower is the microviscosity of the lipid bilayer. The F_e/F_m ratio is therefore inversely proportional to the lipid microviscosity. Fluorescence spectra at 282 nm excitation are used to determine the microviscosity of ring lipids (in the region of protein-lipid contacts) and at 334 nm to determine the viscosity of the lipid bilayer [10, 11].

The polarity of the pyrene microenvironment was estimated by the ratio of the intensity of the first maximum of the pyrene fluorescence spectrum (I_1) to the intensity of the third maximum (I_3) [10].

To determine erythrocyte membrane microviscosity, a heparin – stabilized blood sample taken from a laboratory animal was centrifuged at 3000 rpm (centrifugation: ОПН-8УХЛ4.2 (Dastan, Kyrgyzstan), operating mode 3000 rpm = 930 rcf, $T = 22^\circ\text{C}$) for 10 min. Plasma and upper layer of leukocytes were carefully pipetted and removed. The erythrocytes were washed three times with an isotonic solution containing 0.145 mol/L NaCl in 0.02 mol/L Tris-HCl buffer (pH 7.6 at 20°C), the cells were precipitated each washing round in the same mode (3000 rpm for 10 min). Erythrocyte membranes were obtained by hypoosmotic hemolysis using 0.02 mol/L Tris-HCl buffer (pH 7.6 at 20°C), a volume of washed erythrocytes was rapidly and vigorously mixed with a 2-fold volume of haemolytic solution and incubated for 15 min. Then the mixture was centrifuged at 8000 rpm (centrifugation: ОПН-8УХЛ4.2 (Dastan, Kyrgyzstan), operating mode 8000 rpm = 6600 rcf, $T = 22^\circ\text{C}$) for 15 min. The supernatant was removed, and the membrane precipitate was washed with hypotonic solution of Tris-NS buffer (pH 7.6 at 20°C) until the haemolysate was washed away, each time precipitating the membranes in the same mode [12].

Freshly prepared membrane suspensions were used for fluorescence measurements. Membrane suspension (2 mL) was titrated with 20 μL of 8 $\mu\text{mol/L}$ pyrene alcoholic solution, followed by recording of fluorescence spectra at excitation wavelengths of 282 and 334 nm. The luminescence intensity of pyrene monomers and excimers was determined at 393 and 470 nm (the band maxima), respectively.

3 Results and Discussions

To assess the changes in erythrocyte shape and aggregate formation in pathology and after interaction with nanoparticles, we visually analysed the preparations (erythrocyte suspensions) of collected blood samples using optical micrographs.

Figs. 1a and 1b show microphotographs of erythrocytes from healthy and diseased (liver cancer) rats.

The formation of echinocytes is clearly observed in the blood sample from the diseased animal. It is known that various diseases affect the morphology and aggregation of erythrocytes [13, 14]. The formation of echinocytes (erythrocytes with an altered shape) in liver cancer leads to the decrease in the k coefficient compared to the erythrocytes from healthy rats, while the cell area increases. The corresponding data are shown in the graph (Fig. 2). The observed decrease in the coefficient k depends on the shape of the echinocytes: the larger and sharper are the rays, the larger is the size of the circle circumscribing the cells and the smaller is the inner diameter.

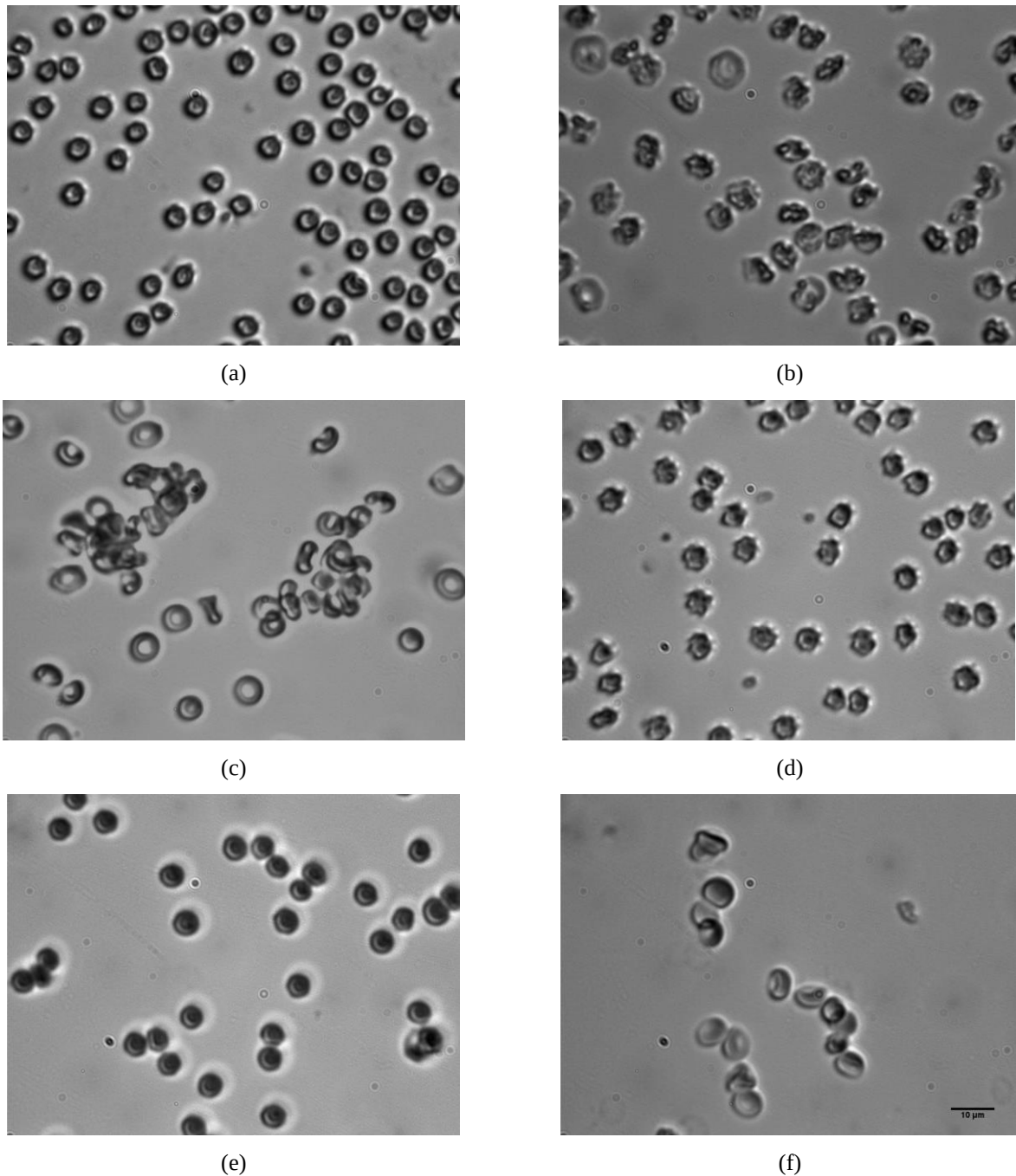


Fig. 1 Microscopic images of the rat erythrocytes samples: (a) healthy animal; (b) diseased animal (DA); (c) (DA) with $\text{NaYF}_4+\text{SiO}_2$ nanoparticles administered; (d) (DA) with NaYF_4+HSA nanoparticles administered; (e) (DA) with $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{folic acid}$ nanoparticles administered; (f) (DA) with $\text{NaYF}_4+\text{HSA}+\text{folic acid}+\text{Cy}_3$ nanoparticles administered.

When nanoparticles are injected into the bloodstream of diseased rats, a change in erythrocyte morphology is also observed. However, the coefficient k increases, *i.e.* the formation of echinocytes decreases. The change in size and shape is the result of interaction between erythrocyte and nanoparticles, which is due to the fact that the nanoparticles are embedded in the outer or inner layer of the membrane [15]. In our case, the nanoparticles are embedded in the outer layer of the membrane, due to the size of the nanoparticles ~ 80 nm, which leads to subsequent membrane stretching and cell shape change.

Calculated from micrographs, the mean area of erythrocytes with NaYF_4 nanoparticles coated with SiO_2 is larger than that of the healthy group ($37 \pm 2.5 \mu\text{m}^2$), but coincides within the error with the control group (diseased rats) ($61 \pm 2.5 \mu\text{m}^2$ and $51 \pm 2 \mu\text{m}^2$, respectively). The coefficient k is comparable to the values of erythrocytes from diseased rats, the change in the morphology of erythrocytes: formation of protuberances, stellate shape, *i.e.* formation of echinocytes is observed in Fig. 1c.

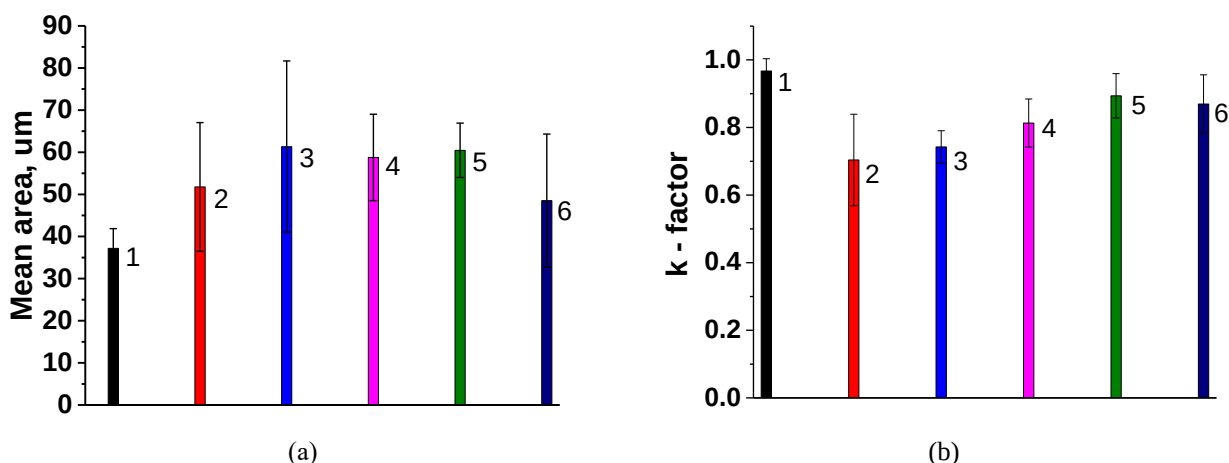


Fig. 2 Results of measurements on microscopic images: (a) mean cell area and (b) mean k-factor of erythrocytes, where erythrocyte preparations: 1) healthy animal; 2) diseased animal (DA); 3) (DA) with $\text{NaYF}_4+\text{SiO}_2$ nanoparticles administered; 4) (DA) with NaYF_4+HSA nanoparticles administered; 5) (DA) with $\text{NaYF}_4+\text{HSA}+\text{FA}$ nanoparticles administered; 6) (DA) with $\text{NaYF}_4+\text{HSA}+\text{folic acid}+\text{Cy}_3$ nanoparticles administered. The calculation is performed for 70 for each of 20 rats.

In addition to the change in shape, strong aggregation is also observed as a result of the interaction, which is a negative indicator for the use of such a coating, as the formation of strong aggregates can be the cause of capillary blockage.

To overcome the problem of aggregation associated with the use of NaYF_4 nanoparticles, we tried an HSA shell for the nanoparticles. It is known [16] that positively charged nanoparticles have haemotoxic effects superior to those of analogous anionic nanoparticles. According to our measurements, the surface potential of NaYF_4 nanoparticles coated with SiO_2 is -0.77 mV, while it is -12.3 eV for nanoparticles with HSA shell. Functionalization of the nanoparticle surface with negatively charged groups cancels the aggregation effect of these nanoparticles on erythrocytes [17].

Since albumin is a blood component (endogenous protein, 35–50 g/L in human serum), it does not show immunogenicity, so such a shell ensures the biocompatibility of the nanoparticles with body cells [18].

After injection of NaYF_4+HSA , as expected, no aggregation was detected, also it was so in the samples with $\text{NaYF}_4+\text{HSA}+\text{FA}$ and $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$. However, in the case of NaYF_4+HSA , the changes in the shape of the erythrocytes were still observed (Fig. 1d), as evidenced by the values of the coefficient k which increased slightly (Fig. 2b).

However, when $\text{NaYF}_4+\text{HSA}+\text{FA}$ or $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$ nanoparticles were introduced into the bloodstream, the change in the shape of erythrocytes (in relation to erythrocytes from healthy animals without nanoparticles) was much less pronounced than in erythrocytes from diseased rats without nanoparticles. The values of the coefficient k of the erythrocytes with nanoparticles coated with albumin, folic acid, and dye are higher than those of the erythrocytes from the diseased rat without nanoparticles

and approach the values of the healthy rat cells without nanoparticles, Fig. 2b.

When nanoparticles coated with albumin-based shells were administered into the bloodstream, the values of the mean area of erythrocytes in the microimages were close within the error (Fig. 2a): for NaYF_4+HSA the mean was $58 \pm 1 \mu\text{m}^2$; for $\text{NaYF}_4+\text{HSA}+\text{FA}$ – $60 \pm 2.5 \mu\text{m}^2$; but for $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$ – $48 \pm 1.5 \mu\text{m}^2$. The values are close to the area values of healthy rat erythrocytes.

In patients with malignant tumours of various localization (lung cancer, gastric cancer, liver cancer), alterations in erythrocyte membranes, lipid bilayer disorders, and changes in the viscosity of intermolecular protein-lipid and lipid-lipid interactions are observed [19]. The interaction of nanoparticles with erythrocyte membrane can also lead to its structural changes in the membrane due to the redistribution of lipids, which is reflected in the microviscosity of the membrane.

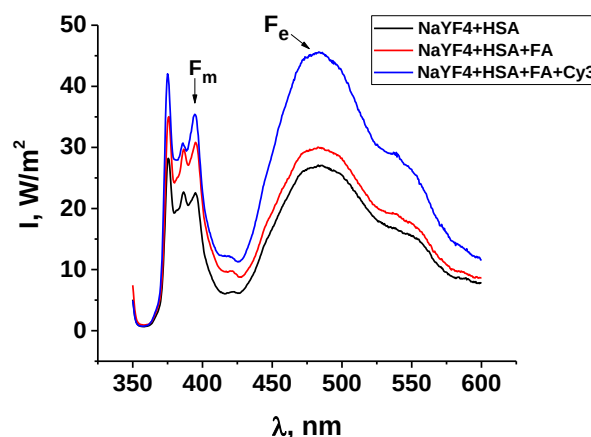


Fig. 3 Fluorescence spectra of pyrene in erythrocyte membranes with different nanoparticles.

Table 1 Spectral characteristics of pyrene fluorescence in erythrocyte membranes with different nanoparticles.

Membranes	F_e/F_m $\lambda_{ex} = 282nm$	I_1/I_3 $\lambda_{ex} = 282nm$	F_e/F_m $\lambda_{ex} = 334nm$	I_1/I_3 $\lambda_{ex} = 334nm$	$(F_0-F)/F_0$
Healthy animal erythrocytes without nanoparticles	1.36 ± 0.07	1.25 ± 0.17	0.57 ± 0.3	1.43 ± 0.16	0.75 ± 0.28
Diseased animal erythrocytes without nanoparticles	2.49 ± 0.42	1.24 ± 0.07	0.92 ± 0.16	1.43 ± 0.07	0.85 ± 0.12
Diseased animal erythrocytes with NaYF ₄ +SiO ₂ nanoparticles	1.3 ± 0.37	1.52 ± 0.13	0.53 ± 0.23	1.64 ± 0.07	–
Diseased animal erythrocytes with NaYF ₄ +HSA nanoparticles	1.57 ± 0.28	1.23 ± 0.04	1.1 ± 0.19	1.22 ± 0.02	0.5 ± 0.033
Diseased animal erythrocytes with NaYF ₄ +HSA+FA nanoparticles	1.25 ± 0.06	1.17 ± 0.03	1.07 ± 0.06	1.2 ± 0.036	0.46 ± 0.0
Diseased animal erythrocytes with NaYF ₄ +HSA+FA+Cy ₃ nanoparticles	2.14 ± 0.02	1.3 ± 0.016	1.33	1.41 ± 0.07	0.56546

Based on the obtained fluorescence spectra of pyrene incorporated into the erythrocyte membrane (Fig. 3), we calculated the degree of excimerization and estimated the polarity of the microenvironment of the pyrene molecules. The results are summarized in Table 1.

Table 1 shows that the differences in the values of F_e/F_m ($\lambda_{ex} = 282nm$) and F_e/F_m ($\lambda_{ex} = 334nm$) ratios for the erythrocyte membranes of diseased and healthy rats were 83% and 61%, respectively. This indicates a strong change in the viscosity of erythrocyte membranes from diseased rats, which affects the change in erythrocyte morphology (shape), as observed in Fig.1b.

When nanoparticles of different surface types are introduced into the bloodstream of diseased rats, the values of the F_e/F_m coefficients ($\lambda_{ex} = 282nm$), measured in the spectra obtained on erythrocyte membrane preparations, decrease by up to 50% compared to the coefficient values for diseased rats without nanoparticle introduction, but are close to the values for erythrocyte membranes from healthy rats.

At the same time, the values of F_e/F_m ($\lambda_{ex} = 334nm$) for NaYF₄+HSA nanoparticles increase by $20 \pm 4\%$ in relation to the values of the coefficient for the diseased rat without introduction of nanoparticles. However, in membrane samples with NaYF₄+HSA+FA+Cy₃ the coefficient increases by 40%, and with NaYF₄+SiO₂ it decreases by 57%. The decrease in the value of the polarity coefficient around the pyrene probe indicates an increase in membrane viscosity and hydrophobicity. Disease has no effect on polarity. Samples with NaYF₄+SiO₂ show the strongest polarity changes at I_1/I_3 ($\lambda_{ex} = 334nm$) and at I_1/I_3 ($\lambda_{ex} = 334nm$).

In the samples with NaYF₄+HSA and NaYF₄+HSA+FA, I_1/I_3 values ($\lambda_{ex} = 334nm$) are 15% lower than in membranes from diseased rats without

nanoparticles, and there is almost no change in I_1/I_3 ($\lambda_{ex} = 282nm$).

Thus, the changes in polarity upon nanoparticle introduction mainly affect lipid bilayer compounds, while ring lipids experience only an upward trend in lipid polarity.

Pyrene eximerization ratio data suggest that nanoparticles affect the microviscosity of erythrocyte membranes:

NaYF₄+SiO₂ promotes an increase in microviscosity (decrease in fluidity) of both total and annular lipids of erythrocyte membranes, as the degree of pyrene eximerization is inversely related to the microviscosity of the lipid phase. The increase in polarity may be due to the hydrophobicity of the NaYF₄+SiO₂ nanoparticle.

NaYF₄+HSA and NaYF₄+HSA+FA contribute to a decrease in microviscosity (increase in fluidity) in the ring lipids of erythrocyte membranes, but contribute to an increase in microviscosity (decrease in fluidity) in total lipids. There is virtually no change in the lipid-protein region and a decrease in polarity in the lipid-lipid region, which may be due to redistribution of pyrene binding to lipids.

NaYF₄+HSA+FA+Cy₃ contributes to a very small decrease in the microviscosity (increase in fluidity) of the ring lipids of erythrocyte membranes, but contributes to an increase in the microviscosity (decrease in fluidity) of the total lipids. The increase in polarity in the lipid-protein region, while there is virtually no change in the lipid-lipid region, may be due to pyrene binding to proteins.

All of the above indicates that due to the interaction of nanoparticles with erythrocyte membranes, there is a decrease in the polarity of the pyrene microenvironment in the lipid region of erythrocyte membranes, which

reduces the fluidity of the lipid bilayer and increases its viscosity. As a result, the membrane becomes harder, is able to keep its shape and does not change, which coincides with the data of the k coefficient calculation and is observed on microphotographs (Fig. 1).

The pyrene probe fluorescence parameter $(F_0-F)/F_0$, which characterizes the efficiency of electron excitation energy transfer from tryptophan residues of membrane proteins to pyrene, shows a significant decrease in the transfer efficiency. The decrease in the parameter $(F_0-F)/F_0$ may indicate structural rearrangements in erythrocyte membrane proteins associated with a decrease in the degree of immersion of proteins in the lipid bilayer or the formation of protein aggregates [20]. The decrease in the degree of immersion of proteins in the membrane lipid bilayer may be associated with an increase in membrane viscosity or changes in the structure of membrane proteins due to their interaction with nanoparticles.

4 Conclusions

The interaction of NaYF₄+SiO₂ nanoparticles with the erythrocyte membrane is accompanied by a change in cell shape and increased formation of aggregates, indicating a change in the properties of the cell

membrane surface. These nanoparticles also affect the state of erythrocytes: both cell size and membrane viscosity.

Nanoparticles coated with albumin without folic acid and albumin with folic acid cause changes in the viscosity of the protein-lipid contact zones of the membrane and the k coefficient tends towards to the value for the healthy rat erythrocytes. The nanoparticles coated with albumin, folic acid, and dye have almost no effect on erythrocytes, both on cell size and membrane viscosity.

The results suggest that the HSA+FA+Cy₃ surface complex of NaYF₄ nanoparticles has better biocompatibility with erythrocytes and that the nanoparticles are embedded in the membrane so that they can be used in photodynamic therapy.

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Disclosures

The authors declare no conflict of interest.

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