

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

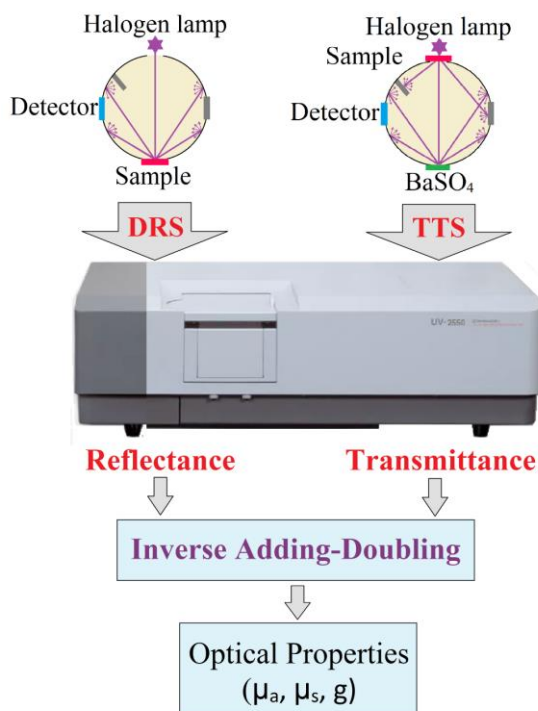


Fig. 1 Schematics of the experimental setup.

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].

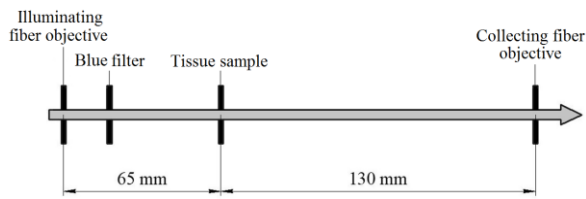


Fig. 2 Schematics of the collimated transmission measurement setup.

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).

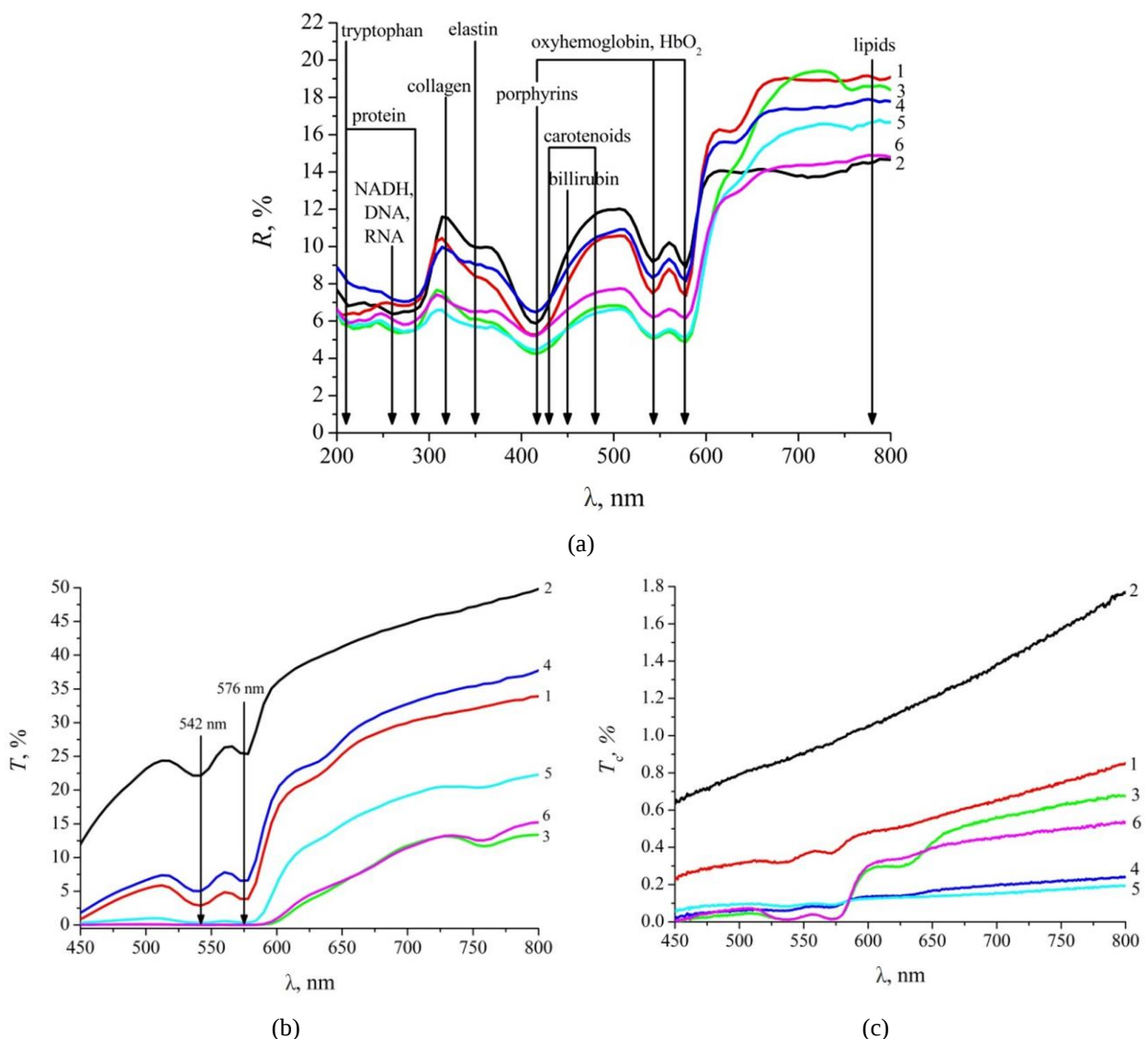


Fig. 3 DRS (a), TTS (b) and T_c (c) of the studied tissues sections of cat ovaries with indicated endogenous chromophores: 1 – leiomyosarcoma, 2 – stromal sarcoma, 3 – serous carcinoma, 4 – granulosa carcinoma, 5 – follicular phase (healthy), 6 – luteal phase (healthy).

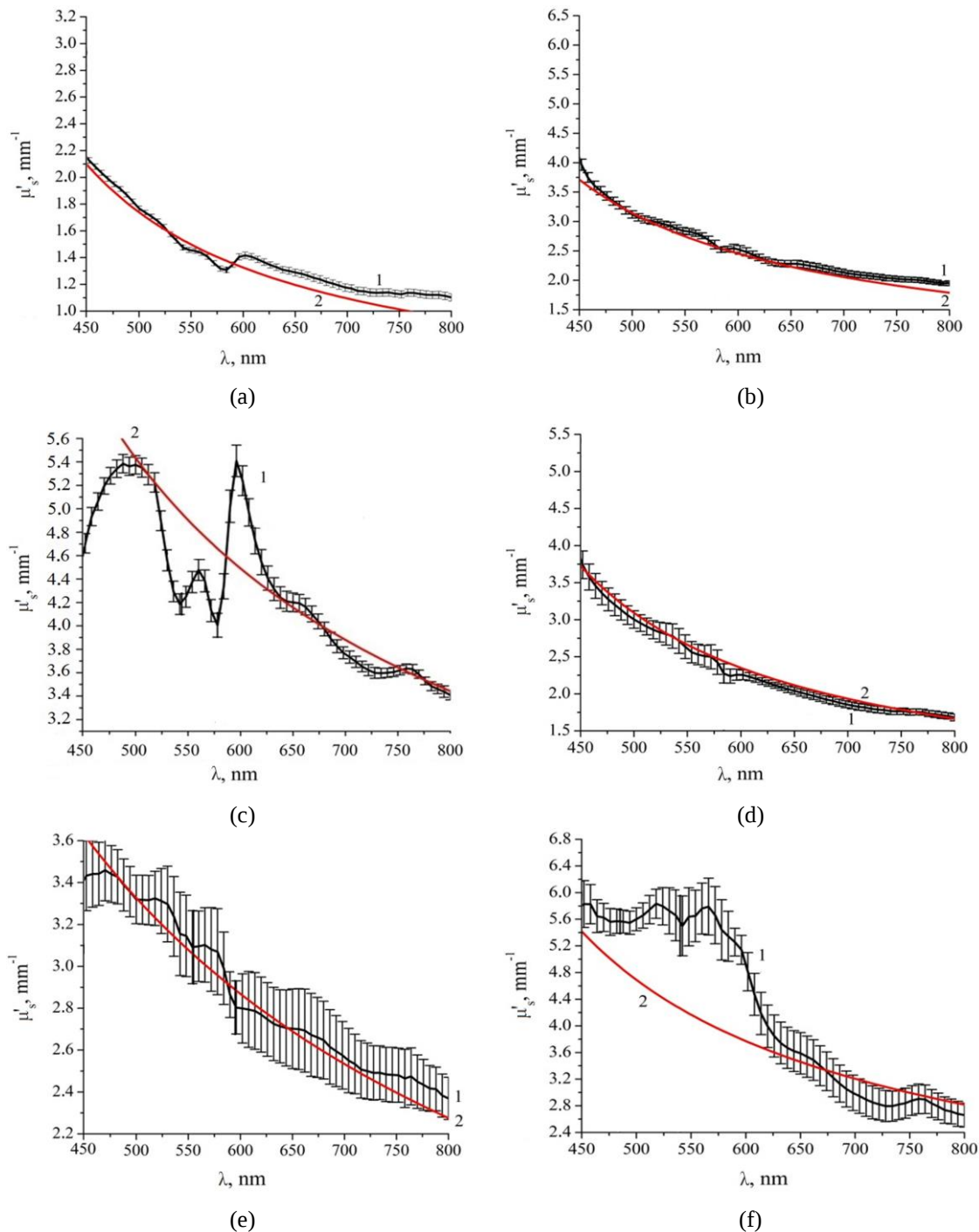


Fig. 4 Spectra of the transport scattering coefficient (1) and approximation graph (2) of the studied cat ovarian samples: (a) leiomyosarcoma (n=5); (b) stromal sarcoma (n=5); (c) serous carcinoma (n=5); (d) granulosa carcinoma (n=5); (e) follicular phase (healthy) (n=5); (f) luteal phase (healthy) (n=5).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

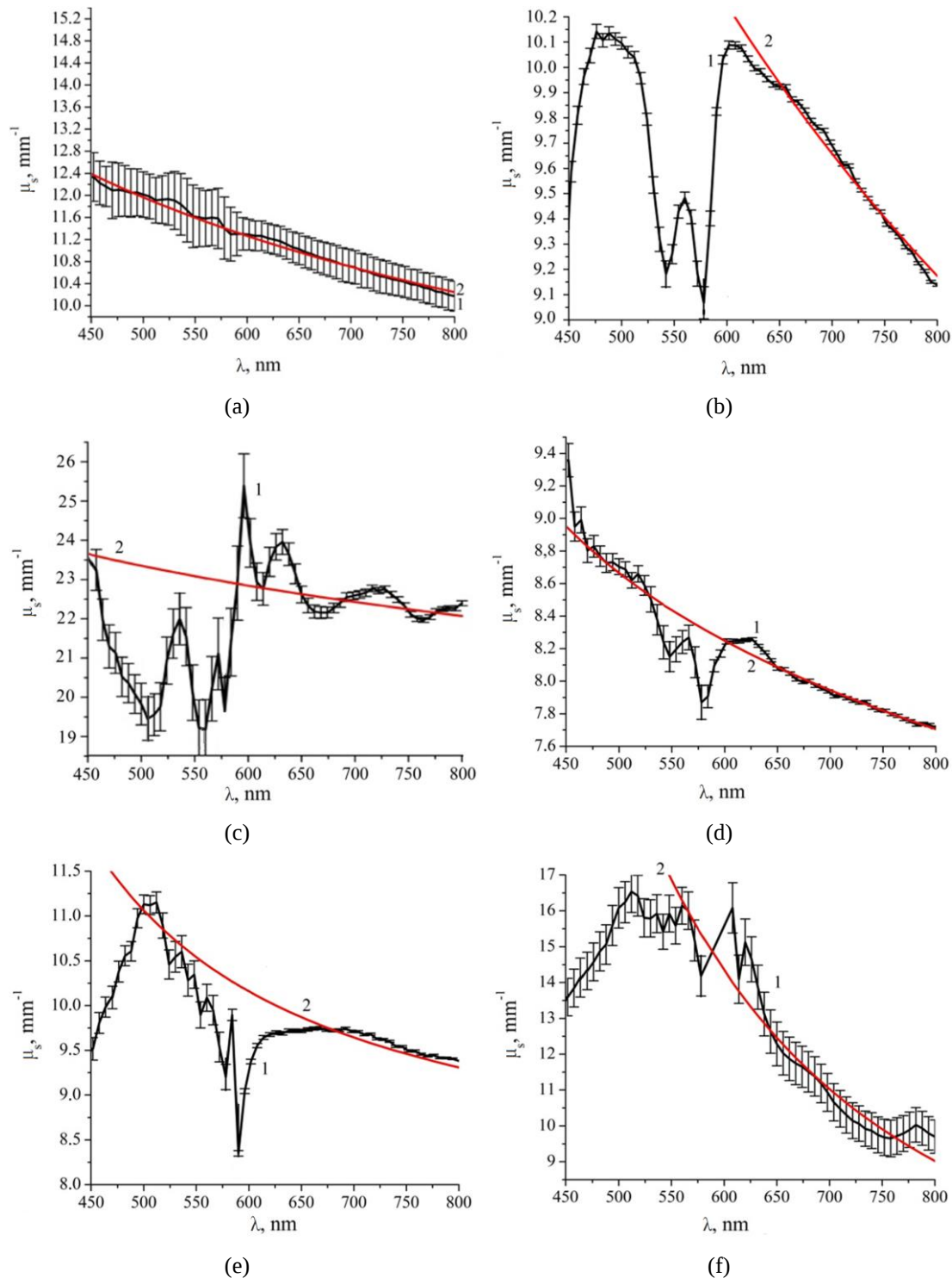


Fig. 5 Spectrum of the scattering coefficient (1) and approximation graph (2) of the ovaries of cats with diagnoses: (a) leiomyosarcoma (n=5); (b) stromal sarcoma (n=5); (c) serous carcinoma (n=5); (d) granulosa carcinoma (n=5); (e) follicular phase (healthy) (n=5); (f) luteal phase (healthy) (n=5).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

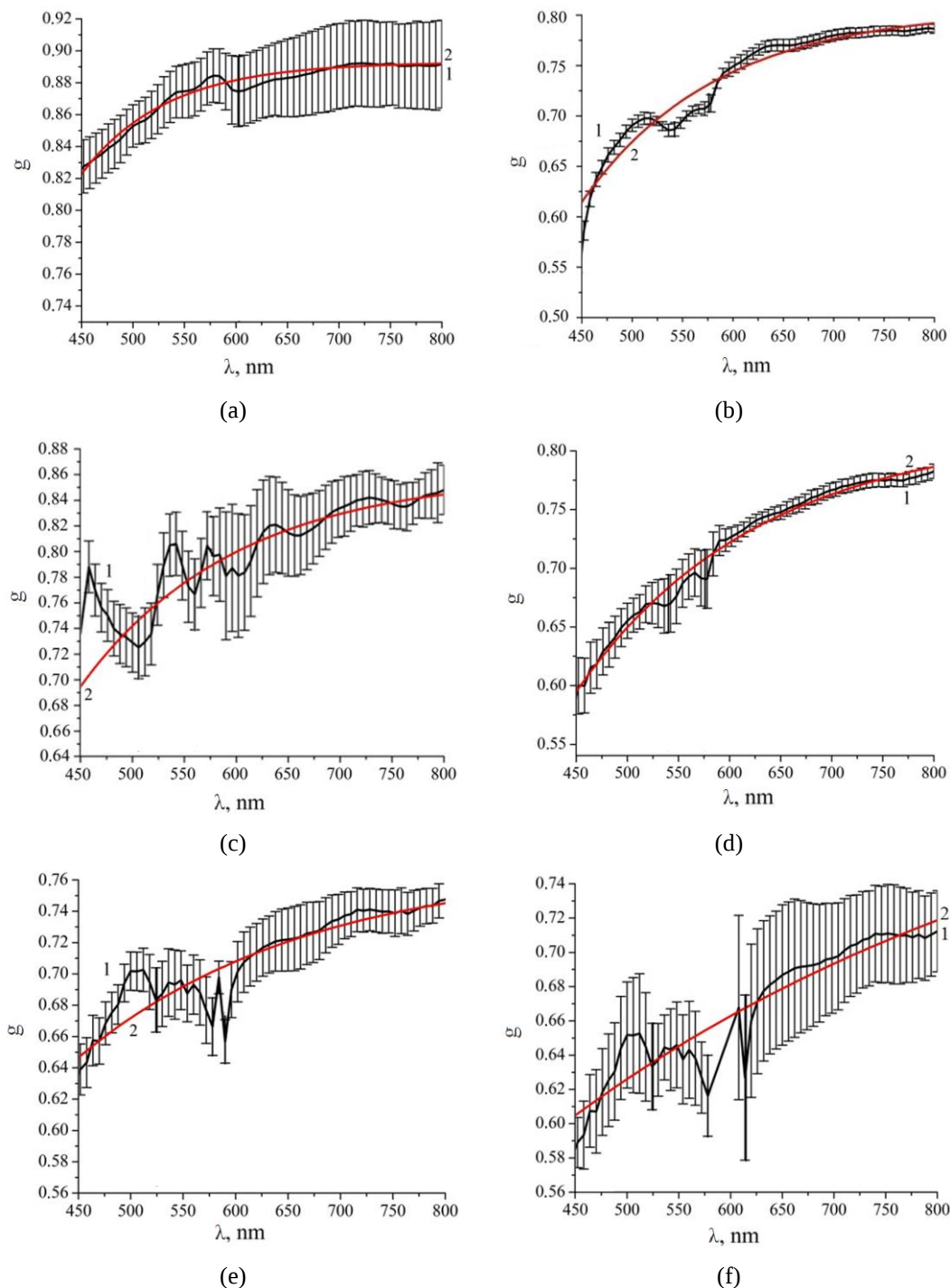


Fig. 6 Spectral dependences of scattering anisotropy factors (1) and approximation graphs (2) of the studied samples of cat ovaries with various diagnoses: (a) leiomyosarcoma ($n=5$); (b) stromal sarcoma ($n=5$); (c) serous carcinoma ($n=5$); (d) granulosa carcinoma ($n=5$); (e) follicular phase (healthy)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

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 leiomyosarcoma, 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).

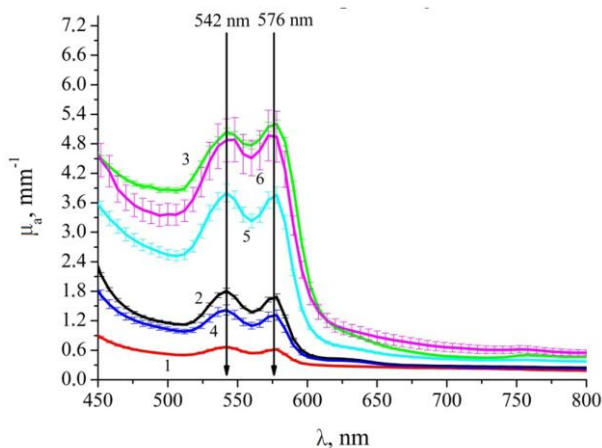


Fig. 7 Absorption spectra reconstructed from DRS and TTS measurements for all studied ovarian samples with various diagnoses: 1 – leiomyosarcoma ($n=5$); 2 – stromal sarcoma ($n=5$); 3 – serous carcinoma ($n=5$); 4 – granulosa cell carcinoma ($n=5$); 5 – follicular phase (healthy) ($n=5$); 6 – luteal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

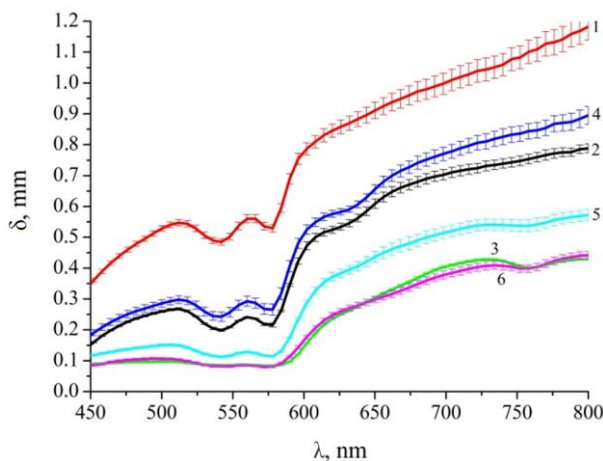


Fig. 8 Dependence of the light penetration depth (δ) into the tissue of the studied ovarian samples with various diagnoses on the wavelength, calculated from the experimental data presented in Figs. 4 and 7: 1 – leiomyosarcoma ($n=5$); 2 – stromal sarcoma ($n=5$); 3 – serous carcinoma ($n=5$); 4 – granulosa 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.

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