

Differences in the Effect of 40%-Glucose on the Optical Properties of Healthy and Stromal-Sarcoma Ovaries in Cats

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Abstract. Differences in diffuse reflectance and total transmittance of healthy and sarcoma cat ovarian tissue were determined using integrating sphere spectroscopy and confirmed by histological analysis. The effective diffusion coefficient determined from the analysis of the kinetics of the diffuse reflectance was found equal to $D = (7.5 \pm 0.9)10^{-7} \text{ cm}^2/\text{s}$ for healthy tissue and $D = (1.4 \pm 0.2)10^{-6} \text{ cm}^2/\text{s}$ for sarcoma. In healthy tissue, optical clearing occurred with the formation of two UV transparency windows: $(18 \pm 4) \text{ nm}$ wide centered at 225 nm and $(50 \pm 12) \text{ nm}$ wide centered at 375 nm. For the ovary with sarcoma, the formation of only one transparency window centered at 380 nm and a width of $(43 \pm 11) \text{ nm}$ was observed. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: ovarian tissues; cancer; glucose; total transmittance spectra; diffuse reflectance spectra; diffusion coefficient; optical clearing efficiency.

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1 Introduction

Ovarian cancer is the fifth leading cause of death for women with cancer worldwide [1]. More than 70% of cancers are only diagnosed at an advanced stage. If ovarian cancer is detected in the initial stages, then the complete remission is 94%, while the detection of a tumor in the later stages reduces the chance of survival to 45% [2]. Overall, the five-year survival prognosis for ovarian cancer remains unfavorable at 46% [3]. The prognosis is closely related to the stage at the time of diagnosis: survival > 70% after 5 years in stage I or II, survival from 20 to 40% in stage III or IV [3, 4]. Despite the advances in modern medicine, the five-year survival of patients with this diagnosis has not increased much over the past half century [4]. Modern research of the problem of early diagnosis and effective treatment of cancer of the reproductive system combines surgical advances, the study of genomics and precision therapy. For example, in advanced economies, patients with ovarian, fallopian tube, or primary peritoneal cancer should undergo genetic risk assessment, and germline and somatic testing. Germline testing refers to the use of

blood or saliva to test for genes that are expected to be identical throughout the body, while somatic testing focuses on genes in a tumor and may affect treatment. In modern clinical practice, it is very difficult to take into account gene mutations in the treatment of specific patients. Interdisciplinary precision medicine research is required because genomic mutations between histological subtypes of ovarian cancer are often observed [5]. Routine ultrasound examination of women does not provide accurate verification of benign and malignant changes [6]. Biological markers are often used to specifically detect epithelial ovarian cancer and metastatic ovarian carcinoma, in particular in the HE4, CA 125, RMI, and ROMA algorithms [7]. To date, the most effective biological diagnostic tool for diagnosing ovarian cancer is the combination of CA125 and HE4 [8]. However, there is no exact probability of detecting malignant changes in the early stages using the above algorithms and tumor markers, since the results are influenced by factors such as smoking or contraception that combines estrogen with progestin, endometriosis, benign changes, etc. [9, 10]. It is important to differentiate early malignant ovarian tumors from benign

ovarian tumors. Therefore, examinations are necessary and should be prioritized in order to advise the patient (monitoring, treatment or examination) according to the lesion, and first of all, taking into account the patient's medical history. Actively developing optical systems may have good prospects for the early detection of cancer, in particular of the reproductive system [11]. However, when using optical systems in clinical medical practice, strong light scattering of biological tissues prevents radiation from passing into deep layers and obtaining ultra-precise images. One of the approaches to solving this problem can be the optical clearing method, which is implemented using optical clearing agents: poly- and monosaccharides, alcohols, dimethyl sulfoxide, etc. [12].

In this work, to realize the main goal of the study, namely, to reliably determine the differences in the optical and diffusion properties of normal and tumor ovarian tissue, 40% aqueous glucose solution was chosen as an optical clearing agent. The objects of the study were clinically healthy ovaries of cats and ovaries with neoplasms in the form of stromal sarcoma, which was confirmed by histological studies. Determination of spectral and kinetic characteristics at optical clearing was carried out in the wavelength range of 200–800 nm by the method of diffusion reflection spectroscopy. Such parameters were determined as the effective diffusion coefficient of 40% glucose in healthy and pathological tissue, as well as the efficiency of optical clearing with finding new virtual windows of tissue transparency.

2 Materials and Methods

For *ex vivo* studies, ovaries from outbred cats aged 5 to 10 years obtained after ovariectomy and ovariohysterectomy in a veterinary hospital were used. A total of 6 samples from 3 ovaries from different cats diagnosed as “clinically healthy” and 6 samples from 2 ovaries from one animal with suspected malignancy

were examined. To clarify the diagnosis, we carried out histological examination of the samples no later than 48 h after removal. To do this, a part of the sample was manually cut 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 taken. For histological examination of all samples, hematoxylin-eosin staining was used. To obtain histological scans, an Aperio AT2 digital slide converter (on-screen diagnostic scanner) equipped with a LED light source and calibration tools was used.

For optical measurements, 6 sections from healthy and 6 sections from pathological ovaries were used. The thickness of tissue sections (samples) was measured with an electron micrometer (Union Source CO., Ltd., China, Ningbo). The measurements were carried out at several points of the sample and averaged. The accuracy of each measurement was ± 0.01 mm. The average thickness of the sections of the ovaries was (0.80 ± 0.09) mm. To measure the diffuse reflectance spectra (DRS) and total transmission spectra (TTS) of tissue samples in the spectral range of 200–800 nm, a Shimadzu UV-2550 double-beam spectrophotometer (Japan, Tokyo) with an integrating sphere was used (Fig. 1).

The light source was a halogen lamp with filtering in the studied spectral range. The limiting resolution of the spectrometer was 0.1 nm. Prior to measurements, the spectra were normalized using a BaSO_4 reference reflector with a suitable reflectivity for the entire spectral range, including UV. All measurements were carried out at room temperature ($\sim 25^\circ\text{C}$) and normal atmospheric pressure. Each sample of the studied tissue was fixed with double-sided adhesive tape in a special frame with a window of 0.5×0.5 cm in a quartz cuvette so that the tissue sample was pressed against the wall of the cuvette and subjected to optical measurement of DRS or TTS, as shown in Fig. 1. The study used 40% aqueous glucose solution (Grotex LLC, Russia, St. Petersburg).

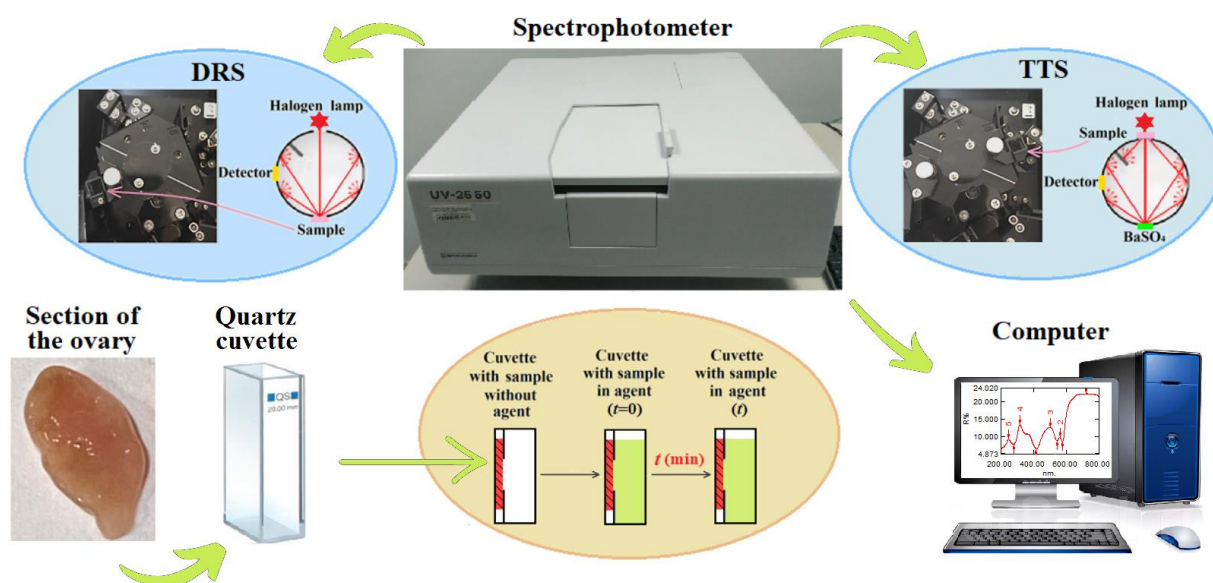


Fig. 1 Scheme of the experimental setup for measuring diffuse reflectance spectra (DRS) and total transmission spectra (TTS) of cat ovarian tissue samples.

For optical measurements, 6 sections from healthy organs and 6 sections from malignant ovaries were used, 3 of which were used to study the kinetics of DRS and 3 to measure TTS.

The protocol of the Local Ethical Committee with permission to conduct animal studies (No. 4 dated 1 November 2022) was issued by the Saratov State Medical University named after V. I. Razumovsky, which is responsible for providing permissions to conduct animal studies for Saratov region.

3 Calculations

The determination of the tissue diffusion coefficient of glucose/interstitial water is based on the measurement of DRS kinetics (Fig. 2). The process of transport of glucose/tissue water in a sample can be described in terms of the free diffusion model [13, 14]. For a plane-parallel tissue sample of a finite thickness, using Fick's second law and the modified Bouguer-Beer-Lambert law, an expression for the difference $\Delta A(t, \lambda)$ between the effective optical density at the current time $A(t, \lambda)$ and at the initial time $A(t = 0, \lambda)$, can be obtained [11–14].

For TTS measurements

$$I(t, \lambda) = I_0 \exp[-\mu_{eff}(t, \lambda)L(\lambda)],$$

where $I(t, \lambda)$ is the transmitted intensity of light, I_0 is the incident intensity of light, and

$$\mu_{eff}(t, \lambda) = \sqrt{3\mu_a[\mu_a + \mu'_s(t)]},$$

t is the time in seconds during which 40%-glucose is applied, λ is the wavelength in nm, $\mu_{eff}(t, \lambda)$ is the effective coefficient of light attenuation in the tissue, 1/cm; $\mu'_s(t) = \mu_s(1 - g)$, 1/cm; g is the scattering anisotropy factor (varies from -1 to 1 , for many

biological tissues, $g \cong 0.93$) [11]; $L(\lambda)$ is the average pathlength of photons, in the transmission mode $L(\lambda) \cong l$, l is the thickness of the sample, cm.

Then,

$$\begin{aligned} \Delta A(t, \lambda) &= A(t, \lambda) - A(t = 0, \lambda) = \mu_{eff}(t, \lambda)L(\lambda) = \\ &= [\mu_{eff}(t, \lambda) - \mu_{eff}(t = 0, \lambda)]L(\lambda) \sim \\ &\sim C_0 \left\{ 1 - \exp\left(-\frac{t}{\tau}\right) \right\} L(\lambda), \end{aligned} \quad (1)$$

where $\Delta\mu_{eff}(t, \lambda)$ is the difference between the effective coefficient of attenuation of light in the tissue $\Delta\mu_{eff}(t, \lambda)$ at the current time and at the initial time, 1/cm;

$$\tau = \frac{4l^2}{\pi^2 D}, \quad (2)$$

D is the diffusion coefficient of the glucose molecules, cm^2/s ; and C_0 is the initial concentration of the glucose, mol/l .

For DRS measurements:

$$A = -\log R(t, \lambda), \quad (3)$$

where $R(t, \lambda)$ is the diffuse reflectance coefficient, and

$$L(\lambda) \cong 2l_d, \quad (l_d)^{-1} = \mu_{eff}l_d.$$

The recorded DRS [$R(\lambda)$, %] are converted using the standard Kubelka–Munk algorithm to optical density $A(\lambda)$ spectra (Shimadzu UV-2550 spectrophotometer software). Glucose is a well-known effective optical clearing agent and is often used in tissues [12, 13]. To evaluate the efficiency of optical clearing of *ex vivo* tissue samples, TTS measurements are usually used, and the efficiency parameter Q is calculated as:

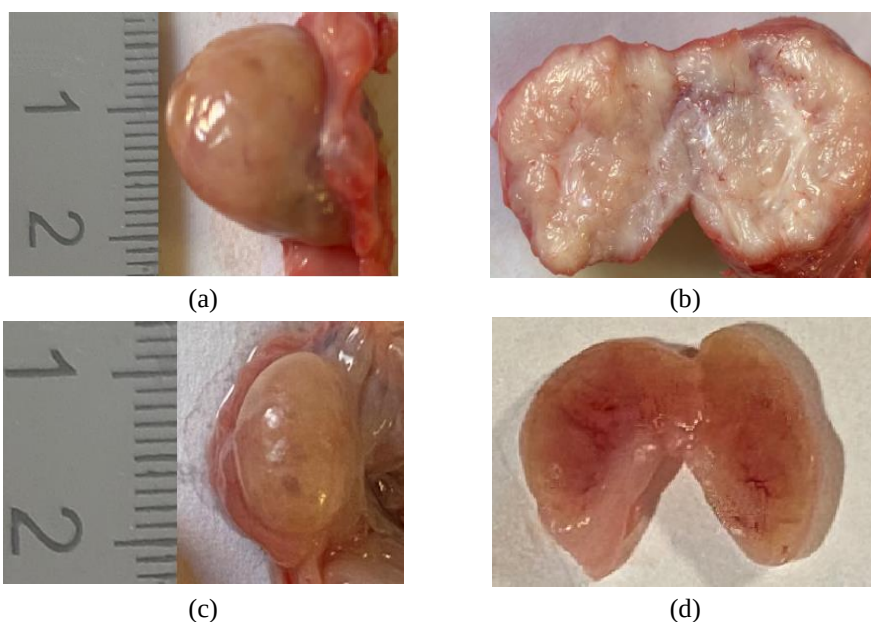


Fig. 2 Photos of the examined ovaries of cats. Whole (a) and in section (b) ovary with stromal sarcoma, whole (c) and in section (d) healthy ovary.

$$Q(\%) = \{T(t, \lambda) - T(t = 0, \lambda)\} / T(t = 0, \lambda), \quad (4)$$

where $T(t = 0, \lambda)$ is the (total transmission coefficient, %) of the tissue sample for a specific wavelength λ at the initial time, and $T(t, \lambda)$ is the same at the current time.

The bars on the DRS and TTS graphs represent the boundaries of the confidence interval, found as:

$$\sigma = (t_s SD) / (\sqrt{n}), \quad (5)$$

where t_s is Student's coefficient, SD is the standard deviation, $n = 3$, and $p = 0,95$.

4 Results and Discussion

4.1 Histological Examination

The histological examination confirmed preliminary clinical diagnoses for ovarian stromal sarcoma tissue (Figs. 3(c, a) and 3(d, b)) and healthy tissue (Figs. 3(c) and 3(d)).

4.1.1 Histology of the Ovary with Stromal Sarcoma

From the outpatient chart: the animal was lethargic, inactive, ate little, and lost weight over the past two months. Over the past three days, the animal's condition has deteriorated significantly, vomiting bile.

The ovary is a large, nonencapsulated, monophasic, highly cellular tumor with aggressive invasive growth. Tumor cells are fusiform, with clear intercellular boundaries, high nuclear-cytoplasmic ratio, homogenous weakly eosinophilic cytoplasm, polymorphic hyperchromic nuclei with finely dispersed chromatin, and 1–2 eosinophilic nucleoli visible at 400 magnification (Fig. 3(a)). The degree of nuclear atypia is high; tumor cells form short intertwining bundles, solid fields. The stroma is poorly developed connective tissue with poor vascularization due to thick-walled vessels of medium caliber of irregular shape. Mitotic activity accounts for 16 mitoses per 10 fields of view at a magnification of 400 (2.37 mm²). Extensive fields of tumor necrosis are determined. Lymphovascular invasion in the volume of the studied material is not determined. A focal moderate polymorphocellular subacute infiltrate is also determined, represented by lymphocytes, plasma cells, macrophages, neutrophils.

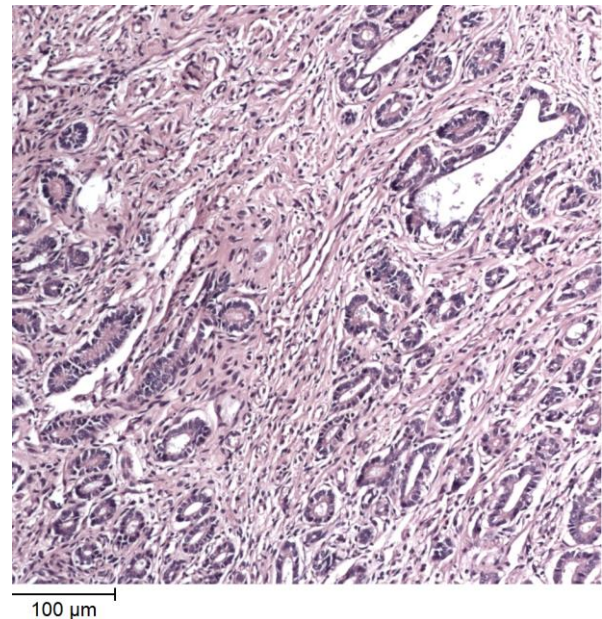
Histological conclusion: the histological picture is most consistent with a malignant mesenchymal tumor, ovarian sarcoma. Given the morphology, stromal sarcoma can be assumed [15].

4.1.2 Histology of Healthy Ovary

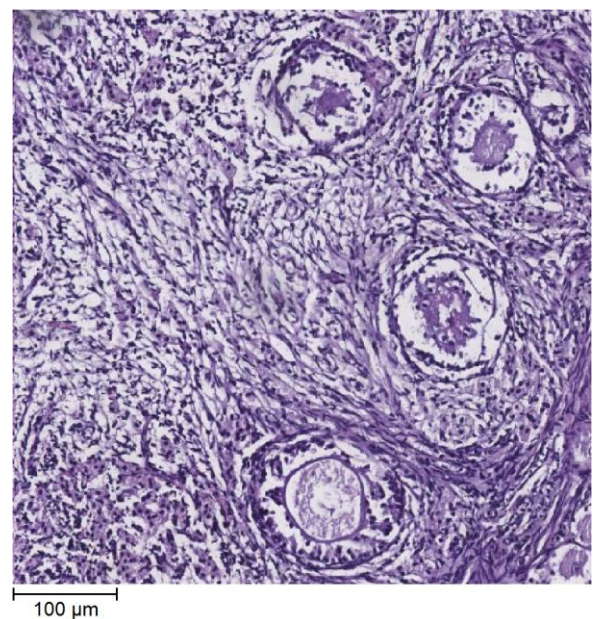
The ovary has a preserved histological structure (Fig. 3(b)). The cortical substance is well developed, prevails over the stroma (2:1), multiple follicles are visualized at different stages of maturation. The corpus luteum is not visualized. Stroma is represented by a typical theca-tissue without edema, poorly developed,

small-focal fresh hemorrhages are noted in it. The vessels are thin-walled, full-blooded.

Conclusion: an ovary with a healthy histological structure.



(a)



(b)

Fig. 3 Photo of a fragment of the histological preparation of the ovary: stromal sarcoma (a), healthy (b).

4.2 Spectrophotometric Studies

DRSs of samples from healthy and malignant ovaries of cats measured before and after interaction with glucose are shown in Figs. 4(a) and 4(c). In the UV range, the initial DRS of ovarian samples have obvious dips characteristic of the absorption bands of amino acid residues of connective tissue proteins in the form of collagen and reticular fibers, hemoglobin, and

porphyrins. In the region of about 415–420 nm and 540–580 nm, the observed dips correspond to the absorption bands of oxyhemoglobin (415, 542, and 576 nm). Water absorption in the measured range of 200–800 nm is insignificant. The diffusion coefficient of glucose/interstitial water in the samples was determined by the least squares method of the experimental curve segment, which characterizes the change in optical density on time of exposure to glucose at selected wavelengths. In Figs. 4(b) and 4(d), the kinetics of DRS during interaction with 40% glucose for 1 healthy and 1 malignant tissue samples are presented. The difference between the effective optical density at the current time and at the initial time ΔA was calculated from experimental data for each sample and then averaged for three the most sensitive to change of scattering wavelengths: 600, 700, and 800 nm.

It can be seen that the interaction of glucose with samples of healthy and malignant ovarian tissue leads to a gradual decrease in the reflectance in the entire studied wavelength range mostly due to decrease of tissue scattering and also some decrease of tissue absorption. Reduction of scattering is provided by refractive index matching of tissue scatterers and interstitial fluid, tissue

dehydration, and better packing of collagen fibers [12, 13]. These effects are caused by two factors: the outflow of intracellular water together with hemoglobin from the tissue due to osmosis and the diffusion of glucose into the tissue (Fig. 5).

The time course of the DRS indicates a decrease in light scattering and, accordingly, makes it possible to unambiguously relate the rate of diffusion of glucose molecules to the rate of change in DRS. Using Eq. (1), we found τ (diffusion time), which for an ovarian tissue samples with sarcoma was 30.0 ± 1.8 min, and for healthy ovarian samples 57.3 ± 3.6 min. In the study of healthy and pathological human colorectal mucosa, similar differences in the values of τ were found, 3.9 min (colorectal carcinoma) and 5.0 min (healthy tissue) [16]. Also diffusion time of 22.3 min was found for glycerol/interstitial water in healthy cat ovarian tissue in follicular phase and 17.7 min – in luteal phase [17].

From experimental data presented in Figs. 4(b) and 4(d), the average diffusion coefficient for 40%-glucose in ovarian samples with sarcoma ($n = 3$) was found as $D = (1.4 \pm 0.2) \cdot 10^{-6}$ cm²/s and for healthy tissue ($n = 3$) as $D = (7.5 \pm 0.9) \cdot 10^{-7}$ cm²/s.

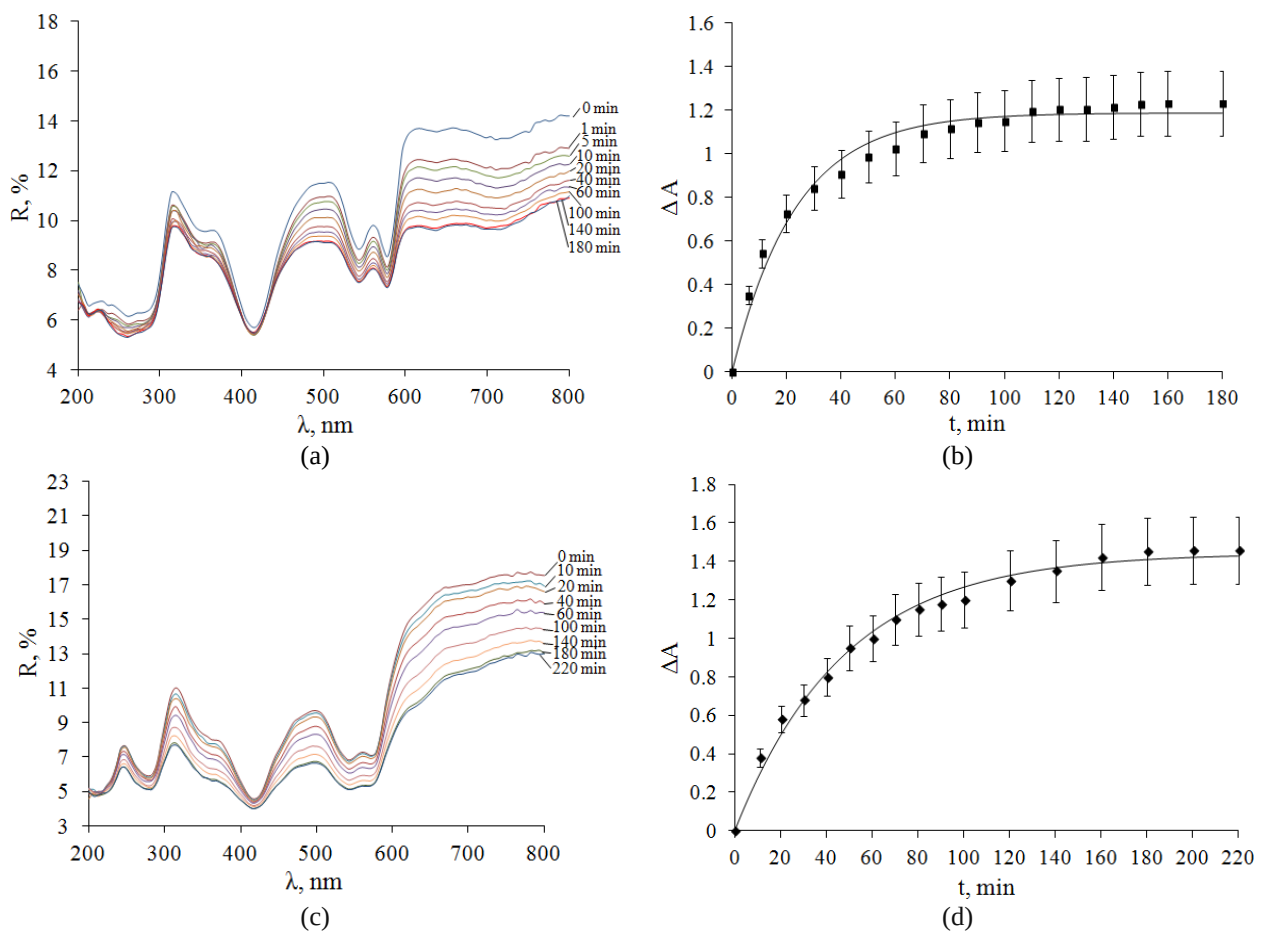


Fig. 4 DRS of cat ovarian tissue samples when immersed in 40% glucose: stromal sarcoma (a), healthy tissue (c). The respective kinetics of the difference in effective optical density $\Delta A(t, \lambda)$ at 600, 700, and 800 nm and then averaged (see Eq. (1)) of the studied ovarian samples. Experimental data marked as symbols, corresponding approximation of experimental data within the framework of the free diffusion model: stromal sarcoma (b), healthy tissue (d) marked as solid curves.

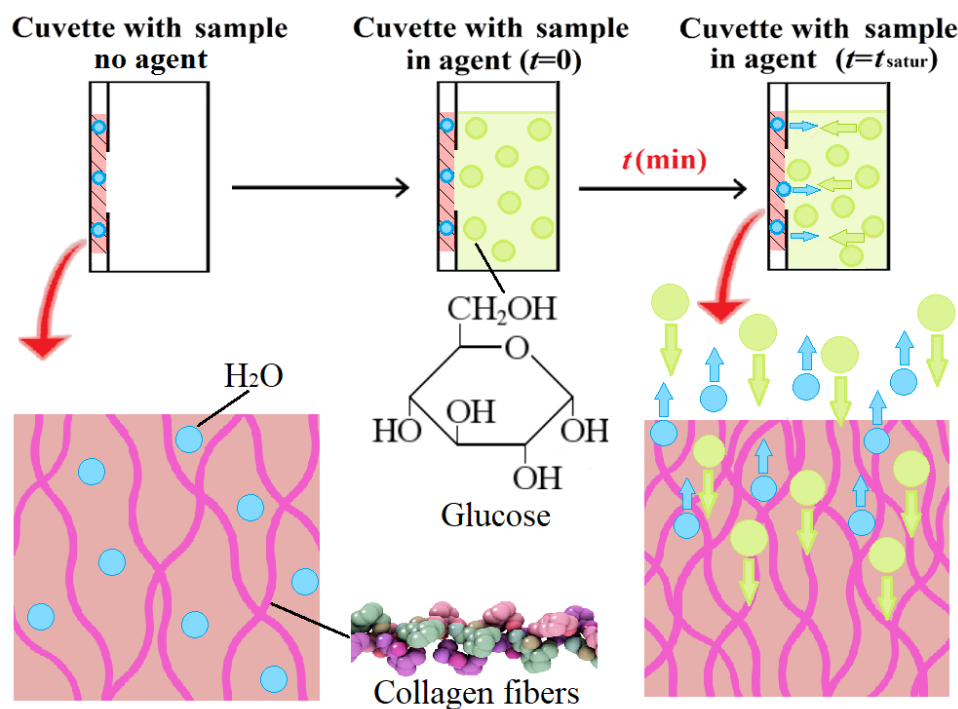


Fig. 5 Diagram showing the interaction of 40%-glucose with ovarian tissue.

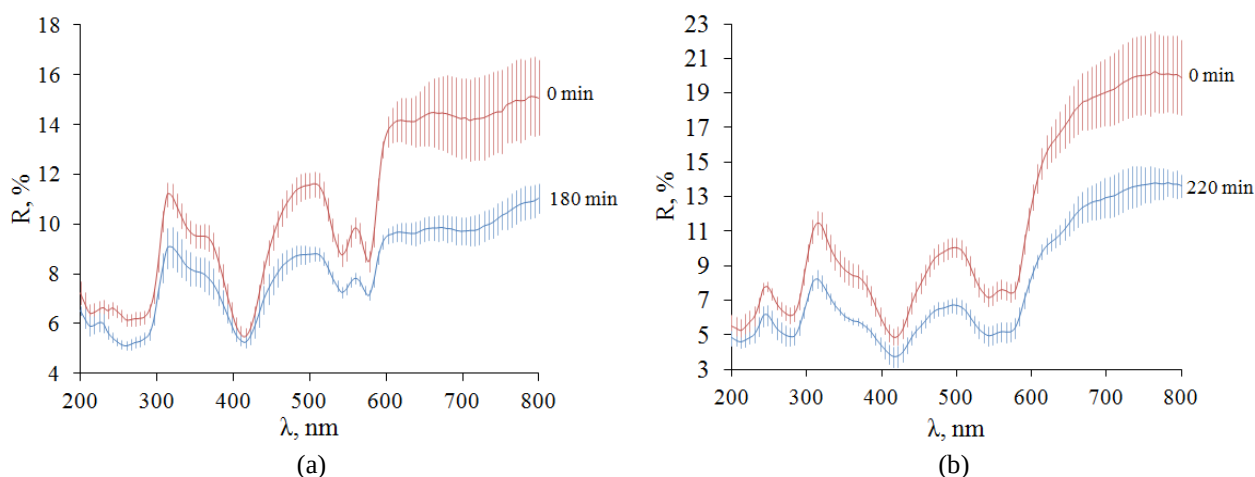


Fig. 6 DRS in the range from 200 to 800 nm of cat ovary tissue before and after immersion in 40 %-glucose: ovaries with sarcoma ($n=6$) (a), healthy ovaries ($n=6$) (b).

The effective diffusion coefficients for glycerol/interstitial water in healthy ovarian samples in follicular phase was determined as $D = (1.9 \pm 0.2) \cdot 10^{-6} \text{ cm}^2/\text{s}$, and in the luteal phase as $D = (2.4 \pm 0.2) \cdot 10^{-6} \text{ cm}^2/\text{s}$ [17], which is expectably faster than for glucose diffusion in healthy tissue due to bigger molecule size in comparison with water and glycerol. The diffusion coefficient for 40%-glucose solution in the healthy gum was determined as $(4.1 \pm 0.8) \cdot 10^{-6} \text{ cm}^2/\text{s}$, which is much higher than in healthy ovarian tissue and explained by difference in structure [18].

DRS of the studied ovary samples were averaged over 6 healthy and 2 malignant samples before and after

interaction with 40% glucose, which are presented in Fig. 6.

TTS of the samples with sarcoma and healthy tissue, before and after interaction with glucose are shown in Fig. 7. In the UV range, the transmission spectra of the samples under study have pronounced dips characteristic to the absorption bands of proteins and blood hemoglobin and correlate with diffuse reflection spectra (Fig. 4 (a, c)).

The total transmittance increases with time over the entire wavelength range with respect to the initial state of the sample (before immersion in glucose), which indicates a decrease in light scattering of the samples as a result of its immersion in glucose.

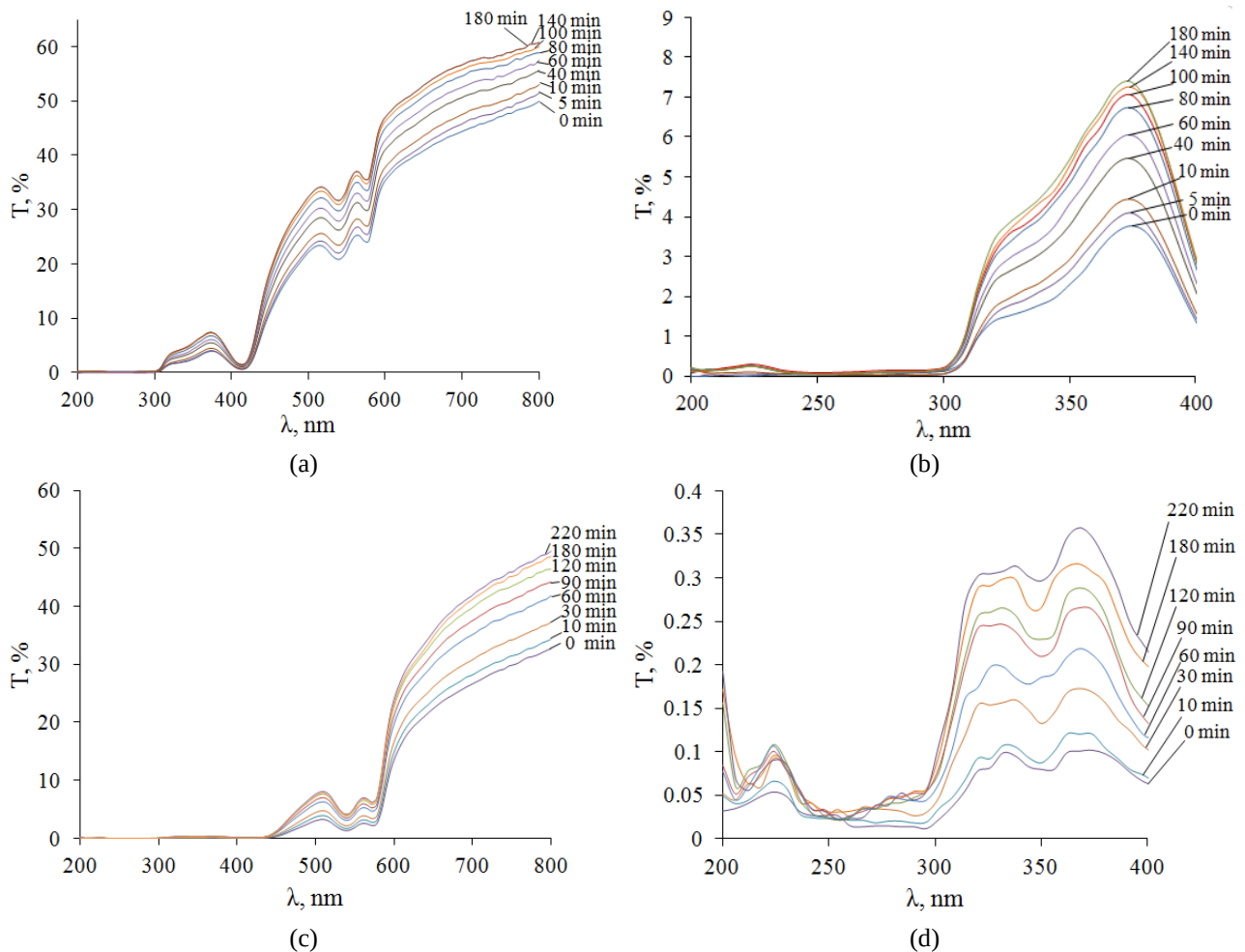


Fig. 7 TTS of cat ovarian tissue samples during immersion in 40%-glucose: an ovary with sarcoma ((a) in the range from 200 to 800 nm, (b) in the range from 200 to 400 nm); a healthy ovary ((c) in the range from 200 to 800 nm, (d) in the range from 200 to 400 nm).

The total transmittance for sarcoma is larger compared to the healthy tissue in the entire studied wavelength range. So, from 300–420 nm, we observe an increase in T from 3.9% to 7.4% after 180 min of interaction of the pathological tissue with 40%-glucose. For healthy tissue, T in the entire UV region does not exceed 0.1% before interaction and 0.36% after interaction with 40%-glucose. In the case of pathological tissue, the absolute values of the transmittance vary from 22.2% to 32.4% (at 500 nm), from 35.8% to 47.4% (at 600 nm), from 44.3% to 56.6% (at 700 nm), and from 50.1% to 60.8% (at 800 nm). In the case of healthy ovarian tissue, the transmittance varies from 3.2% to 7.6% (at 500 nm), from 14.2% to 25.0% (at 600 nm), from 26.5% to 41.7% (at 700 nm), and from 32.7% to 49.6% (at 800 nm). Thus, 40%-glucose is an effective optically clearing agent [12].

In Fig. 8, ovary TTS averaged over 6 healthy and 2 malignant samples before and after interaction with 40% glucose are presented.

After completing immersion of the samples, it can be seen that in healthy tissue, optical clearing occurred with the formation of two UV transparency windows: (18 ± 4) nm wide centered at 225 nm and (50 ± 12) nm wide centered at 375 nm (Figs. 8(a) and 8(b)). For the

ovary with sarcoma, the formation of only one transparency window centered at 380 nm and a width of (43 ± 11) nm was observed (Figs. 8(c) and 8(d)). The efficiency of optical clearing of biological tissue Q is an important characteristic of the interaction of tissue with an OCA. Using experimental data and Eq. (4), parameter Q was calculated for 40%-glucose action on healthy and sarcoma ovarian tissues (Fig. 9).

In healthy ovarian samples, the efficiency of optical clearing was about 260% in the range from 200–450 nm, up to 290% at 450 nm, 160% at 500 nm, 230% at 550 nm; no more than 80% from 600 to 800 nm. In ovarian samples with sarcoma, optical clearing efficiency is no more than 100% from 200 to 450 nm, and no more than 50% from 500 to 800 nm. Similar results were obtained when using highly concentrated glycerol for the optical clearing of healthy colorectal tissues and in polyposis pathologies, as well as healthy gingival tissue [19].

In the literature, there are a large number of studies of various diseases and areas of interest in the human body, conducted using animal tissue samples [20, 21]. The effectiveness of the study of the reproductive system of cats has been shown using the ultrasound method [22] and X-ray with contrast agents [23],

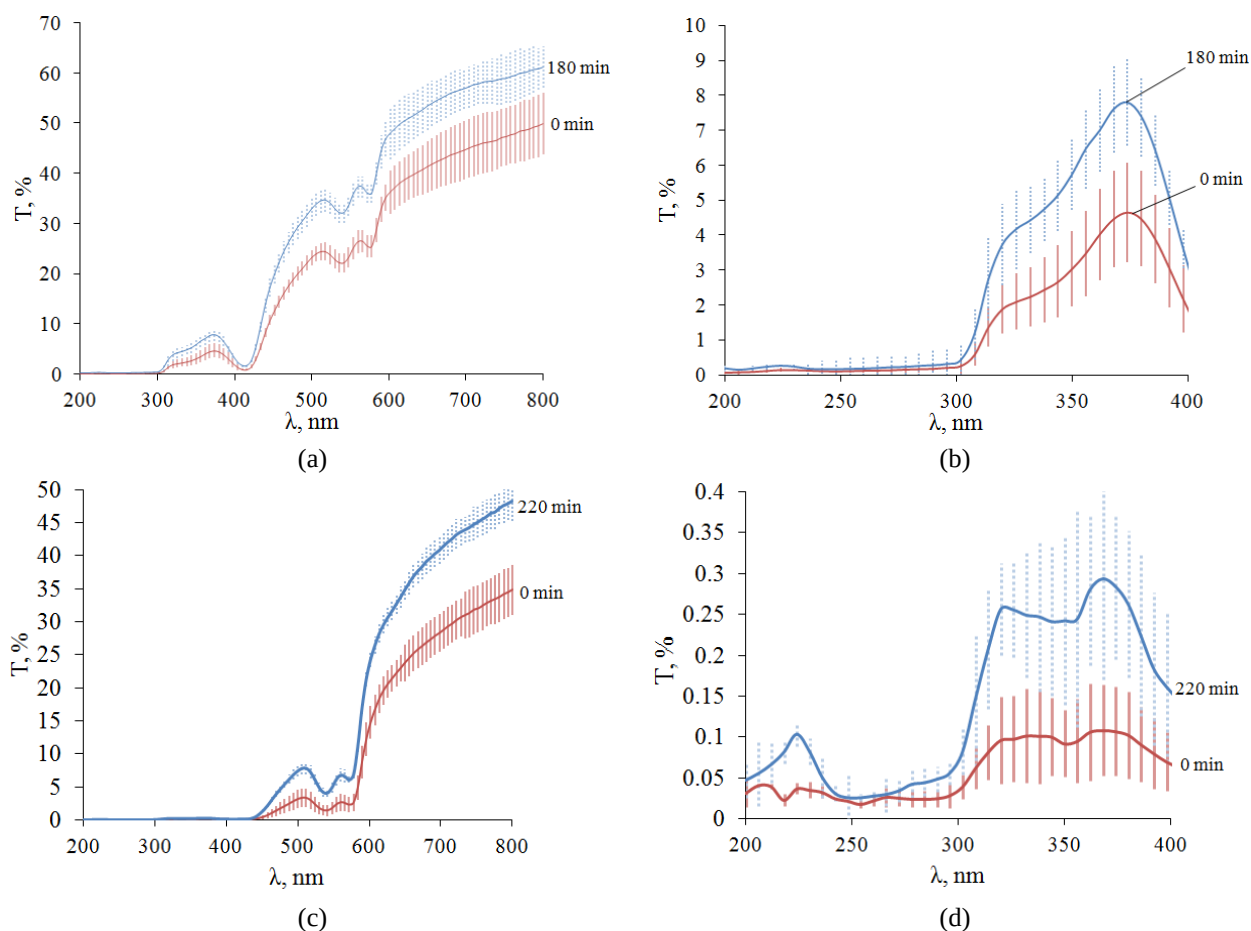


Fig. 8 Averaged TTS of cat ovary tissue before and after immersion in 40%-glucose: spectra of ovaries with sarcoma ($n=6$) from 200 nm to 800 nm (a), from 200 nm to 400 nm (b), spectra of healthy ovaries ($n=6$) from 200 nm to 800 nm (c), from 200 nm to 400 nm (d).

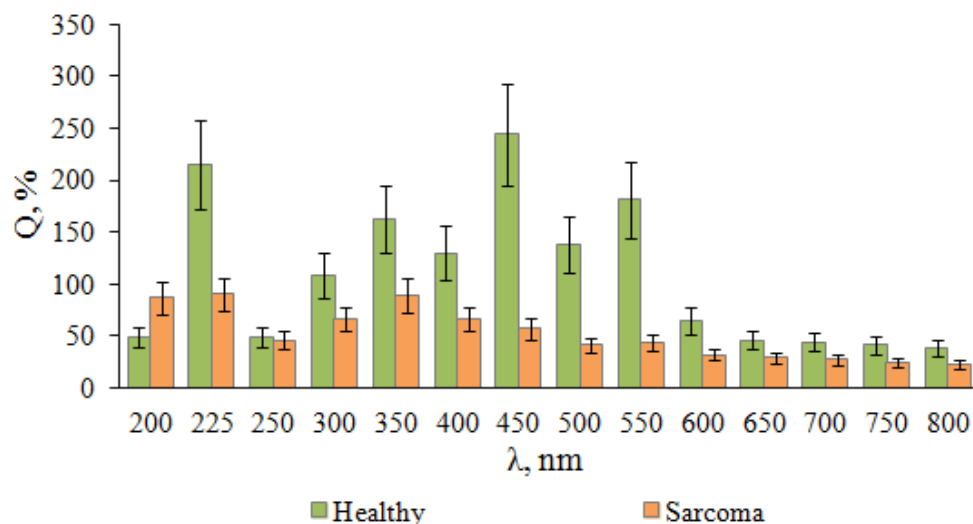


Fig. 9 Efficiency (Q , %) of tissue optical clearing after exposure to 40%-glucose during 180 min for stromal sarcoma and 220 min for healthy tissue.

the neuroanatomy of the brain of cats was studied using the methods of cryosection, magnetic resonance imaging and computed tomography [24], nuclear medicine application refers to a method in which radioactive compounds are injected into an animal to be

examined, followed by imaging and measurement of the radiation emitted [25], etc. However, there are a lack of optical methods for studying tissues of the reproductive system of domestic mammals, in particular, cats and

dogs, which indicates the relevance of the ongoing research.

5 Conclusion

Differences in the perfusion-kinetic parameters of the healthy ovaries and with sarcoma at 40%-glucose action were revealed. The diffusion coefficient was determined as $D = (7.5 \pm 0.9) \cdot 10^{-7} \text{ cm}^2/\text{s}$ for healthy tissue and $D = (1.4 \pm 0.2) \cdot 10^{-6} \text{ cm}^2/\text{s}$ for stromal sarcoma. Glucose diffusion coefficient in the tumor ovarian tissue is greater than in the healthy tissue, which is due to bigger content of free water and the ability of the malignant tissue to induce uptake of glucose as an energy for growth.

For healthy tissue, in the UV region two virtual windows were found, one with a center at 225 nm and $(18 \pm 4) \text{ nm}$ wide and another centered at 375 nm and $(50 \pm 12) \text{ nm}$ wide. In the ovary samples with the stromal

sarcoma, the formation of a single transparency window in the UV spectral region with a center at 380 nm and a width of $(43 \pm 11) \text{ nm}$ was observed. The results obtained allow us to recommend 40%-glucose as an effective agent for optical clearing, which can be used in clinical practice at topical application.

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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.

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