Capability of physically reasonable OCT-based differentiation between intact brain tissues, human brain gliomas of different WHO grades, and glioma model 101.8 from rats

I. N. Dolganova,1,2,* P. V. Aleksandrova,1 P. V. Nikitin,3,4 A. I. Alekseeva,1,5 N. V. Chernomyrdin,4 G. R. Musina,4 S. T. Besplav,3,4 I. V. Reshetov,6,7 A. A. Potapov,3 V. N. Kurlov,1,2 V. V. Tuchin,8,9,10 and K. I. Zaytsev2,4

1Institute of Solid State Physics of the Russian Academy of Sciences, Chernogolovka 142432, Russia
2Institute for Regenerative Medicine, Sechenov First Moscow State Medical University (Sechenov University), Moscow 119991, Russia
3Burdenko Neurosurgery Institute, Moscow 125047, Russia
4Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow 119991, Russia
5Research Institute of Human Morphology, Moscow 117418, Russia
6Institute for Cluster Oncology, Sechenov First Moscow State Medical University (Sechenov University), Moscow 119991, Russia
7Academy of Postgraduate Education FSCC FMBA, Moscow 125310, Russia
8Saratov State University, Saratov 410012, Russia
9Institute of Precision Mechanics and Control of the Russian Academy of Sciences, Saratov 410028, Russia
10Tomsk State University, Tomsk 634050, Russia

* dolganova@issp.ac.ru

Abstract: Optical coherence tomography (OCT) of the ex vivo rat and human brain tissue samples is performed. The set of samples comprises intact white and gray matter, as well as human brain gliomas of the World Health Organization (WHO) Grades I–IV and glioma model 101.8 from rats. Analysis of OCT signals is aimed at comparing the physically reasonable properties of tissues, and determining the attenuation coefficient, parameter related to effective refractive index, and their standard deviations. Data analysis is based on the linear discriminant analysis and estimation of their dispersion in a four-dimensional principal component space. The results demonstrate the distinct contrast between intact tissues and low-grade gliomas and moderate contrast between intact tissues and high-grade gliomas. Particularly, the mean values of attenuation coefficient are 7.56±0.91, 3.96±0.98, and 5.71±1.49 mm⁻¹ for human white matter, glioma Grade I, and glioblastoma, respectively. The significant variability of optical properties of high Grades and essential differences between rat and human brain tissues are observed. The dispersion of properties enlarges with increase of the glioma WHO Grade, which can be attributed to the growing heterogeneity of pathological brain tissues. The results of this study reveal the advantages and drawbacks of OCT for the intraoperative diagnosis of brain gliomas and compare its abilities separately for different grades of malignancy. The perspective of OCT to differentiate low-grade gliomas is highlighted by the low performance of the existing intraoperative methods and instruments.

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1. Introduction

Optical coherence tomography (OCT), proposed at the end of the 20th century [1–3], has found its wide application in biology and medicine, since it provides fast, noninvasive, label-free imaging of surface and internal structure of tissues [4–7]. Today, OCT systems can be rather
compact, including combination with small probes, endoscopes, and needles [4,8], demonstrate subcellular-level resolution [9–12] compared to ultrasound and magnetic resonance imaging (MRI) [13], and can be effectively combined with other imaging modalities, such as confocal microscopy [14] and fluorescent imaging [15], or even terahertz pulsed spectroscopy and imaging [16].

Since OCT enables imaging contrast between healthy and malignant soft tissues relying mainly on changes of optical scattering properties, it became a promising instrument in oncology [17]. Particularly, the abilities of OCT for brain tumor imaging and detection are being extensively studied, aimed at the solution of an urgent neurosurgical problem of gross total resection of tumors along with preservation of healthy tissues [18]. This problem is accompanied with a strong infiltrative character of such tumors as glioma, the infiltrative region of which can not be imaged by the intraoperative MRI [19] and fluorescent-guided metabolic navigation using 5-aminolevulinic acid (5-ALA) in case of low-grade glial tumors [20–23]. In addition, various reactive and inflammatory processes contribute to the accumulation of a fluorescent agent outside the tumor cells, which can significantly complicate the identification of the true tumor margins [24].

The label-free nature of OCT contrast between different brain tissue types is based on a wide combination of tissue properties, including the presence and organization of myelin fibers, layered or bulk structure of tissues, presence of hemorrhages, necrosis, microcalcifications, cysts, vascularizations, cell and nuclear density, nuclei sizes, content and state (free or bound) of tissue water, etc. All of them more or less alter the optical properties of tissue and the corresponding changes of OCT signal. Therefore, much attention should be paid to the conditions of measured samples. For example, formalin fixation of the ex vivo tissue specimens significantly alters the OCT signal [25], but even in this case the differences between various tissue types can be observed [26,27]. On the other hand, it was shown that strong dehydration of brain tissues also should be prevented by cooling and covering of samples, for instance by glass [28]. Moreover, in vivo and ex vivo OCT measurements feature different contrast and signal intensity for different parts of brain mainly due to the presence or absence of blood perfusion [29]. However, the exact impact of each of the possible morphological, chemical, and physical properties on OCT signal has not been entirely clarified yet.

The direct interpretation of “raw” OCT images of brain tissues is rather subjective, partly due to the aforementioned complex origin of OCT signal. Thus, an extraction of specific parameters is often applied. Estimation of the attenuation coefficient, particularly using the single-scattering approximation, is the most common approach [30–35]. Attenuation coefficient was measured for rodent and human in vivo and ex vivo brain tissues and demonstrated abilities to distinguish different tissue types. However, the distinguishing between human glioma and normal cortex tissues is a challenging problem, though in animal models such differences are often observed [34]. Nevertheless, a comparison between the values of attenuation coefficients reported by various research groups demonstrates strong variance, caused additionally by differences of the applied OCT modalities, sample preparation procedures, measurement conditions, and signal processing routine. These facts even more extend the initial dispersion of measured parameters originated from the natural variance of tissue properties.

Besides the attenuation coefficient, the recent research of the texture-based classification analysis combined with machine learning methods [36,37] and speckle contrast analysis [35] have demonstrated rather promising results but need to be tested at larger sample set. However, the connection of the texture-based and speckle intensity parameters with physical and structural properties of tissue samples still need to be analyzed. In addition, such OCT-based techniques and modifications as optical frequency domain imaging [38], and polarization-sensitive OCT [28,34,39–42] demonstrate high potential to differentiate brain tissues, though they demand significant technical modifications of the equipment, which increases its cost. In this work, we
suggest extracting several physically reasonable features of OCT signal using clinically applied endoscopic OCT systems, and estimate the impact of their variance on possible capabilities of differentiation between intact tissues and tumors.

For this aim, OCT of the ex vivo malignant and intact brain tissue samples of humans and rats was performed, including human gliomas of the World Health Organization (WHO) Grades I–IV [43] and glioma model 101.8 [44,45]. Attenuation coefficient and normalized peak of reflection intensity (the latter is a function of the tissue refractive index) using the reference glass, and the standard deviations of these parameters were studied. The ability to differentiate pathological tissues from intact white matter and cortex was estimated, applying a statistical analysis based on the principal component analysis for the extracted values of parameters. The results of our study demonstrated a strong variance of the obtained values, which is enlarged for higher glioma Grades.

This research allows for systematical analysis of the contrast between intact brain and tumor tissue properties, both of humans and rats, and observation of the moderate differences between them. Particularly, the mean values of attenuation coefficient are 7.56±0.91, 3.96±0.98, and 5.71±1.49 mm$^{-1}$ for human white matter, glioma Grade I, and glioblastoma, respectively. Concerning the endogenous nature of this contrast, the results confirm the prospects of OCT for the intraoperative neurodiagnosis, especially in case of low-grade gliomas, when the existing methods and instruments for tissue differentiation demonstrate low performance. Meanwhile, the revealed variance of the analyzed parameters obstructs the diagnosis of the high-grade (III–IV) human brain gliomas, which is confirmed by the observed high false positive and false negative errors of tissue differentiation. Thus, the transferring of OCT approach into neurosurgical practice requires the further improvements, particularly the development of the appropriate methods and protocols of signal processing and data analysis.

2. Materials and methods

2.1. Tissue samples

Diffuse gliomas are the second of the most common types of tumors of the Central Nervous System (CNS). According to the 2016 revision of WHO classification of CNS tumors [43], there are the following nosological types:

- Grade I, i.e. benign astrocytic tumor including pilocytic astrocytoma and subependymal giant cell astrocytoma;

- Grade II, i.e. diffuse astrocytic tumor including diffuse astrocytoma with/without a mutation in the genes isocitrate dehydrogenase 1 ($IDH1$) or 2 ($IDH2$), gemistocytic astrocytoma, oligoastrocytoma, oligodendroglioma;

- Grade III, i.e. diffuse astrocytic tumor including anaplastic astrocytoma with/without a mutation in the $IDH1$ or $IDH2$ genes, anaplastic oligoastrocytoma, anaplastic oligodendroglioma;

- Grade IV, i.e. diffuse astrocytic tumor including glioblastoma of different nosological types (glioblastoma with/without mutation in the $IDH1$ or $IDH2$ genes, epithelioid glioblastoma, giant cell glioblastoma, and gliosarcoma).

The present study included analysis of rat and human ex vivo brain tissue samples. The measured human brain samples are listed in the Table 1. They were excised during neurosurgical operations, according to the initial medical diagnosis. Intact tissues were obtained when accessing the tumors. OCT imaging was performed no later than 4 hours after surgery. Wistar rats with and without glioma model 101.8 [44,45] were also included in the experiment. The rat brain samples were cut into two parts and the frontal ones were measured by the OCT no later than
10 minutes after excision. Further, comparing the rat OCT data with histological images, the A-scans, which corresponded to the cortex (4197 scans), white matter (1832 scans), and glioma model (1820 scans), were picked for the detailed analysis.

### Table 1. Human brain tissue samples.

| #  | Gender | Age | Tissue type / Pathology          | WHO Grade | Number of A-scans |
|----|--------|-----|---------------------------------|-----------|-------------------|
| 1  | M      | 66  | perifocal cortex                | –         | 1410              |
| 2  | F      | 69  | perifocal cortex                | –         | 724               |
| 3  | M      | 45  | perifocal white matter          | –         | 2280              |
| 4  | M      | 38  | perifocal white matter          | –         | 2310              |
| 5  | F      | 39  | pilocytic astrocytoma           | I         | 405               |
| 6  | F      | 31  | pilocytic astrocytoma           | I         | 1700              |
| 7  | F      | 25  | gangliocytoma                   | I         | 4690              |
| 8  | M      | 29  | diffuse astrocytoma             | II        | 3170              |
| 9  | F      | 63  | diffuse astrocytoma             | II        | 3350              |
| 10 | M      | 31  | diffuse astrocytoma             | II        | 140               |
| 11 | M      | 43  | oligodendroglioma               | II        | 2890              |
| 12 | F      | 56  | oligodendroglioma               | II        | 3655              |
| 13 | F      | 47  | anaplastic astrocytoma          | III       | 2720              |
| 14 | F      | 26  | anaplastic oligodendroglioma    | III       | 2710              |
| 15 | M      | 41  | anaplastic glioma               | III       | 200               |
| 16 | F      | 69  | glioblastoma                    | IV        | 1650              |
| 17 | M      | 52  | glioblastoma                    | IV        | 6439              |
| 18 | F      | 64  | glioblastoma                    | IV        | 6800              |
| 19 | F      | 69  | glioblastoma                    | IV        | 4613              |
| 20 | F      | 55  | glioblastoma                    | IV        | 1650              |
| 21 | F      | 20  | glioblastoma                    | IV        | 5160              |

During the transportation before the measurements, the samples were cooled and covered by the gelatin films, which were removed only after the experiment. Such gelatin-embedding of tissue prevents from its hydration-dehydration and sustains the optical properties unaltered for a couple of hours [46,47]. The measured brain tissue samples were placed on a reference optical glass (Fig. 1). After collecting OCT images, all tissues were fixed with formalin for the further Hematoxylin and Eosin (H&E)-stained histology and confirmation of the initial diagnosis. During the measurements (10–20 min depending on the sample size), the position of OCT probe was fixed by the camera for better correlation with further histopathological results.

### 2.2. Analysis of OCT images

For the experimental study, we use the endoscopic OCT system OCT1300Y from the Institute of Applied Physics RAS (Nizny Novgorod, Russia) [48,49]. This OCT employs a laser radiation with the central wavelength of 1.3 µm and the average power of 0.6 mW. It yields B-scans (2D depth images of 256×400 pixels) of the sample with optical penetration depth near 1.0 mm and depth resolution of 24 µm in air by scanning range 1.93 mm in lateral direction with lateral resolution of 20 µm. This system was developed for clinical applications and thus contains an endoscope for measuring hardly-accessible tissues and internal organs, but also suffers from lower resolution and lower lateral scanning range, compared to ones used in scientific workflows. The region of interest for each image consists of 380 A-scans; 20 A-scans are ignored in order to avoid
Fig. 1. OCT imaging of brain tissues; (a) the measurement arrangement and (b) OCT image; (c) and (d) example of an A-scan and extraction of (c) attenuation coefficient and (d) normalized peak of reflection intensity.

Aside distortion. For the described below analysis, no averaging of A-scans is performed. In our opinion, studying capabilities of such endoscopic OCT systems in intraoperative neurodiagnosis, considering their limited resolution, sensitivity and scanning range is of high practical importance for real-life applications. Figure 1(c) and (d) demonstrate a typical A-scan $I(z)$ in the experimental scheme for a particular vertical cross-section of the sample. The intensity peaks $I_1$ and $I_2$ correspond to the reflection from the interfaces the OCT probe – reference window and reference window – the sample, respectively.

In our research, the four OCT signal parameters were introduced, i.e.

- The attenuation coefficient $\mu$, by power, for A-scan in the single-scattering approximation [50–52], which exponentially reduce the OCT signal intensity as

  \[ I(z) = I_0 \sqrt{R(z)}, \]  
  \[ R(z) = C \exp(-2z\mu), \]  

where $z$ is an optical depth, $I_0$ is the maximal signal intensity corresponding to the reference window – sample interface, $R(z)$ is sample reflectance, and $C$ denotes the parameter depending on the back-scattering coefficient and system point spread function (PSF) [52]. In optical range, attenuation coefficient of tissues is mainly determined by a scattering coefficient, since the absorption is significantly lower [53]. In order to estimate $\mu$, one can apply linear fit of the measured $I(z)$ in logarithmic representation for the depth region between the position $z_2$ of second intensity peak and the depth $z_{\text{noise}}$, that denotes the signal attenuation to noise level (Fig. 1(c)). When the determination of $z_{\text{noise}}$ is not obvious, the minimization of mean square error can be performed

  \[ z_{\text{noise}} = \arg \min_z \left[ \frac{\sum_{z \geq z_{\text{noise}}} [I(z) - I_{\text{fit}}(z, \mu)]^2}{N_{\text{decay}}} + \frac{\sum_{z \geq z_{\text{max}}} [I(z) - I_{\text{noise}}]^2}{N_{\text{noise}}} \right], \]  

where $I_{\text{fit}}(z, \mu)$ is the sloped fit line, $I_{\text{noise}} = I_{\text{fit}}(z_{\text{noise}}, \mu)$ is the horizontal fit line corresponding to noise level, $z_{\text{max}}$ is the maximal depth of the sample, $N_{\text{decay}}$ and $N_{\text{noise}}$ correspond to the numbers of terms in the first and second sums, respectively. The analysis of $\mu$ reflects mostly the structural properties of tissue, such as the cell and nuclei density and size, the presence and organization of myelin fibers, etc.
• Normalized peak of reflection intensity of the A-scan (Fig. 1(d))

\[ K = \frac{I_2}{I_1} \propto \left( \frac{1 - n_2/n_1}{1 + n_2/n_1} \right)^2, \]  

(4)

which is defined by the variable effective refractive index of tissue \( n_2 \) and the fixed refractive index of the reference glass \( n_1 \). Since \( K \) depends on the effective refractive index of a sample, its analysis helps to pay attention on the dielectric properties of tissue, which depends on its chemical composition, calcification, the state and concentration of water and other components [46,52,54,55].

• Standard deviation of \( \mu \) within the specific region in the lateral direction around the A-scan, which includes \( N_A \) neighboring A-scans, i.e.

\[ \sigma_\mu = \sqrt{\frac{1}{N_A} \sum_{i=1}^{N_A} (\mu_i - \bar{\mu})^2 / N_A} \right]^{0.5}, \]

(5)

where \( \bar{\mu} \) is the mean value of \( \mu \) in the estimated region, which size corresponds to 150 \( \mu m \) (the typical size of small vessel in a brain tissue).

• Standard deviation of \( K \) within the same region of 150 \( \mu m \) in the lateral direction around the A-scan

\[ \sigma_K = \sqrt{\frac{1}{N_A} \sum_{i=1}^{N_A} (K_i - \bar{K})^2 / N_A} \right]^{0.5}, \]

(6)

where \( \bar{K} \) is the mean value of \( K \) in the estimated region.

The two latter parameters \( \sigma_\mu \) and \( \sigma_K \) directly depend on the small-scale heterogeneity of tissue, which can be caused by the possible vascularization or the presence of hemorrhages, necrosis, and cysts, and can effectively add the estimation of \( \mu \) and \( K \), making the analysis of brain tissues more thoroughly.

3. Results

3.1. OCT imaging of the rat brain tissues

Figure 2 demonstrates the OCT study of the ex vivo rat brains. Samples were scanned along several lines by the OCT probe, and the obtained OCT images were used for the analysis of parameters \( \mu, \sigma_\mu, K, \) and \( \sigma_K \). From the “raw” OCT images shown in this Figure, the visual similarity of the cortex and tumor regions is clearly observed, particularly, their signal intensity decays less strong than in white matter and seems to be more homogeneous. These facts are confirmed by the values of the analyzed parameters shown in the panels (f)–(m) of Fig. 2.

Figure 3(a) and (b) demonstrate the distribution of these parameters for all analyzed OCT images, whereas panels (c) and (d) demonstrate distribution of the same parameters obtained for Intralipid aqueous solutions. 8, 16, and 32% aqueous solutions of initial 20% Intralipid suspension (Fresenius Kabi, Germany) were applied for calibration of OCT system, using the previously measured values of \( \mu \) [56,57]. 10 normalized measurements of each concentrations form the blue regions in (c) and (d) by means of the proposed algorithm of parameters extraction; the centers of these regions define mean values, while their widths stand for standard deviations and describe the instrumental variance of data, i.e. PSF of our endoscopic OCT system. Note that they are significantly smaller than the dispersion of measured tissue parameters, thus, the observed dispersion OCT data for tissues can be attributed to the natural variability of their optical properties. It should be mentioned that at this moment, it is rather difficult to estimate the
Fig. 2. OCT imaging of the ex vivo rat brains; (a),(d) H&E-stained histology of the sample sections; (b),(e) the examples of “raw” OCT images of the white-marked regions from the scanning lines; (f),(i) \( \mu \); (g),(k) \( \sigma_\mu \); (h),(l) \( K \); (i),(m) \( \sigma_K \); values from (f),(g),(h),(i) are obtained for the scanning line from (a), values from (j),(k),(l),(m) – for the scanning line from (d); cortex regions are colored in blue, white matter – in green, tumor – in red.

exact relation between \( K \) and tissue refractive index due to its complexity and dependence on system PSF [52], thus, the calibration was implemented only for \( \mu \). This particular problem is the scope of our future study, but in the present work, it is beyond our attention.

Characterizing the parameters of rat brain tissues, the arrays of points on the \( \mu \)-diagram (Fig. 3(a)) can be split out, oppositely, the points on the \( K \)-diagram (panel (b)) are overlapped. Thus, attenuation coefficient seems to be more informative for distinguishing ex vivo rat brain tissue types. But one should pay attention that \( \sigma_\mu \), as well as \( \mu \), brings valuable information about tissues for their possible differentiation. Since \( \sigma_\mu \) is higher for white matter, this tissue is more heterogeneous than tumor and cortex, i.e. possesses inclusions with dimensions around 150 \( \mu \)m and lower.

In order to estimate the relative distributions of the observed values between different tissue types, a statistical analysis was applied, i.e. some kind of a linear discriminant analysis [58,59], which uses the projections of each data points from two arbitrary tissue types on a line through their mean values \( P_G \), \( P_{WM} \) or \( P_C \) (see Fig. 3(a)). The resulted histograms are demonstrated in Fig. 3(e)–(j). It is observed that the \( \mu \)-histogram of white matter has small overlap with \( \mu \)-histograms of cortex (panel (g)) and tumor (panel (e)), but according to these data, the distinguishing between cortex and tumor is slightly possible (Fig. 3(f)). \( K \)-histograms in panels (h)–(j) demonstrate the smaller width of tumor distribution than of healthy tissues, possibly due to the artificial nature of glioma model. However, for rat brain tissues, \( \mu \)-parameters are more informative than \( K \)-parameters.

The main statistical information about the measured data is shown in Table 2. Its first part includes the mean values of the parameters \( \mu \), \( \sigma_\mu \), \( K \), and \( \sigma_K \), as well as their standard deviations. The changes of attenuation coefficient obtained in our study (i.e. \( 5.89 \pm 1.22 \) mm\(^{-1} \)) for white
Fig. 3. Analysis of the OCT imaging of rat brains samples \textit{ex vivo}; (a), (b) are the distributions of $\mu$ vs $\sigma_{\mu}$ and $K$ vs $\sigma_{K}$, respectively; (c), (d) calibration of the present OCT system using Intralipid aqueous solutions and data from Refs. [56, 57], blue areas correspond to the experimental distribution of measured 10 images of each solution normalized in $\mu$-axis at the referenced data; (e)-(g), (h)-(j) are the corresponding differentiation histograms, obtained from the analysis of (a) and (b) distributions, respectively. Here, WM stands for white matter, C – cortex, G – glioma model, $P_i$ stands for the position of the mean value of points distribution for $i$-th tissue type, LDA space stands for linear discriminant analysis space, which is the line between $P_i$ central points.

matter, $2.72 \pm 1.13$ mm$^{-1}$ for cortex, and $2.39 \pm 0.75$ mm$^{-1}$ for glioma model) are in good agreement with the results from Ref. [42], (i.e. median values $9.39$ mm$^{-1}$, $2.25$ mm$^{-1}$, $3.72$ mm$^{-1}$, respectively). While the variance of values may be caused by different OCT systems, as well as measurement and calculation approaches, the rat white matter features significantly higher $\mu$ than cortex and tumor model, which demonstrate rather similar values of attenuation. Using the histograms from Fig. 3 and set the border between tumorous and non-tumorous distributions equal to the middle of $P_GP_{WM}$ or $P_GP_C$ lines (see Fig. 3(a)), the false positive (FP) and false negative (FN) errors were analyzed, see the second part of Table 2. It also confirms the minimal errors ($FP=0.08$, $FN=0.02$) for distinguishing between white matter and glioma model in rat brain. Note, the estimation of the boundaries between distribution of different tissue types is beyond the scope of our study, therefore the simple criterion of two classes differentiation was applied.

3.2. OCT Imaging of the human brain tissues

The same OCT analysis, as described above in the Subsection 3.1, was performed for the \textit{ex vivo} human brain samples. The results are demonstrated in Figs. 4, 5, 6, and 7 and summarized in Table 3. In these figures, the distributions of the analyzed parameters of different glioma Grades are compared with cortex and white matter, their mean values and standard deviations are shown in the first part of Table 3. Note, the considered samples of perifocal white matter and cortex from Table 1 were used together in the analysis, thus, the distributions of points of these tissues are similar in Figs. 4–7. As it can be noticed from the last column of Table 3 and panels (d) of
Table 2. Results of the rat brain measurements.

| Tissue type       | mean(µ), mm⁻¹ | mean(σµ), mm⁻¹ | mean(K) | mean(σK) |
|-------------------|----------------|----------------|---------|----------|
| white matter (WM) | 5.89 ± 1.22    | 2.38 ± 1.02    | 0.87 ± 0.14 | 0.15 ± 0.07 |
| cortex (C)        | 2.72 ± 1.13    | 0.80 ± 0.56    | 0.81 ± 0.12 | 0.14 ± 0.07 |
| glioma model 101.8 (G) | 2.39 ± 0.75 | 0.88 ± 0.53    | 0.74 ± 0.06 | 0.13 ± 0.06 |

Tissue types FP, µ-space FN, µ-space FP, K-space FN, K-space

WM vs G 0.08 0.02 0.40 0.16
C vs G 0.46 0.39 0.46 0.32

Figs. 4, 5, 6, and 7, white matter and cortex have different values of σK – namely, 0.10 and 0.16, respectively, which reveals different heterogeneity of their refractive index; and these two types of tissues can be distinguished on the K-diagram (green and blue arrays of points), oppositely to the data obtained for the rat brain tissues.

![Image](image_url)

**Fig. 4.** Analysis of the OCT imaging of human brain samples ex vivo; (a) an OCT image of glioma Grade I tissue, (b) the representative histological image; (c),(d) distributions of [µ, σµ] and [K, σK], respectively; (e)-(f), (g)-(h) differentiation histograms between glioma Grade I (G) and intact tissues (cortex (C) and white matter (WM)), obtained from panels (c),(d), respectively. LDA stands for linear discriminant analysis space.

In case of gliomas of Grade I (Fig. 4) and Grade II (Fig. 5), according to the obtained results, tumorous and intact tissues have different properties, and even the analysed values of cortex can be distinguished from those of low-grade gliomas. Their mean values of µ for Grade I and II (3.96 mm⁻¹ and 3.66 mm⁻¹ , respectively) are almost two times lower than those for perifocal intact tissues. The moderate differences of σµ are observed for Grade I and II (2.01 mm⁻¹ and 2.04 mm⁻¹), comparing with the value of cortex (2.33 mm⁻¹). This is reflected in the relatively low FP and FN errors calculated in µ-space. The minimal errors among all the analyzed combinations of tissue types were obtained for white matter compared with Grade II, FP=0.02 and FN=0.01. In addition, only the slight split of histograms of K-parameters is observed. Thus, the differentiation is better for the analysis of µ-parameters.
Fig. 5. Analysis of the OCT imaging of human brain samples \textit{ex vivo}; (a) an OCT image of glioma Grade II sample, (b) the representative histological image; (c),(d) distributions of $[\mu, \sigma_\mu]$ and $[K, \sigma_K]$, respectively; (e)-(f), (g)-(h) differentiation histograms between glioma Grade II (G) and intact tissues (cortex (C) and white matter (WM)), obtained from panels (c),(d), respectively. LDA stands for linear discriminant analysis space.

Fig. 6. Analysis of the OCT imaging of human brain samples \textit{ex vivo}; (a) an OCT image of glioma Grade III sample, (b) a representative histological image; (c),(d) distributions of $[\mu, \sigma_\mu]$ and $[K, \sigma_K]$, respectively; (e)-(f), (g)-(h) differentiation histograms between glioma Grade III (G) and intact tissues (cortex (C) and white matter (WM)), obtained from panels (c),(d), respectively. LDA stands for linear discriminant analysis space.
Fig. 7. Analysis of the OCT imaging of human brain samples ex vivo: (a) an OCT image of glioma Grade IV sample, (b) a representative histological image; (c),(d) distributions of $[\mu, \sigma_\mu]$ and $[K, \sigma_K]$, respectively; (e)-(f), (g)-(h) differentiation histograms between glioma Grade IV (G) and intact tissues (cortex (C) and white matter (WM)), obtained from panels (c),(d), respectively. LDA stands for linear discriminant analysis space.

Table 3. Results of the human brain measurements.

| Tissue type       | $\text{mean}(\mu)$, mm$^{-1}$ | $\text{mean}(\sigma_\mu)$, mm$^{-1}$ | $\text{mean}(K)$ | $\text{mean}(\sigma_K)$ |
|-------------------|-------------------------------|--------------------------------------|-------------------|--------------------------|
| white matter (WM) | 7.56 ± 0.91                   | 1.99 ± 0.85                          | 1.03 ± 0.09       | 0.10 ± 0.03              |
| cortex (C)        | 6.39 ± 1.20                   | 2.33 ± 1.16                          | 1.02 ± 0.11       | 0.16 ± 0.05              |
| glioma Grade I (GI) | 3.96 ± 0.98                 | 2.01 ± 0.88                          | 1.06 ± 0.09       | 0.13 ± 0.04              |
| glioma Grade II (GII) | 3.66 ± 0.75                | 2.04 ± 0.92                          | 0.97 ± 0.12       | 0.13 ± 0.07              |
| glioma Grade III (GIII) | 7.30 ± 1.21             | 2.58 ± 1.02                          | 0.97 ± 0.09       | 0.14 ± 0.05              |
| glioma Grade IV (GIV) | 5.71 ± 1.49              | 2.06 ± 1.01                          | 1.07 ± 0.17       | 0.16 ± 0.08              |

| Tissue types | FP, $\mu$-space | FN, $\mu$-space | FP, $K$-space | FN, $K$-space |
|--------------|-----------------|-----------------|--------------|----------------|
| WM vs GI     | 0.03            | 0.02            | 0.35         | 0.37            |
| WM vs GII    | 0.02            | 0.01            | 0.33         | 0.32            |
| WM vs GIII   | 0.22            | 0.38            | 0.27         | 0.24            |
| WM vs GIV    | 0.11            | 0.27            | 0.26         | 0.52            |
| C vs GI      | 0.16            | 0.12            | 0.29         | 0.33            |
| C vs GII     | 0.14            | 0.05            | 0.47         | 0.35            |
| C vs GIII    | 0.34            | 0.32            | 0.48         | 0.36            |
| C vs GIV     | 0.44            | 0.39            | 0.34         | 0.53            |
The distribution of $\mu$-parameters of glioma Grade III (Fig. 6) looks rather different than those of Grades I and II and slightly can be distinguished from intact tissues, which is confirmed by high FP and FN errors. Its mean attenuation coefficient $\mu = 7.3 \text{ mm}^{-1}$ significantly exceeds those of low Grades and approaches that of white matter. However, its dispersion is the highest among the analyzed tissues, $\sigma_\mu = 2.58 \text{ mm}^{-1}$ and the $K$-parameters are similar to those of Grade II.

The analysis of glioma Grade IV properties (Fig. 7) reveals their high dispersion. Grade IV features the highest standard deviation of mean $\mu$ and $K$, 1.49 mm$^{-1}$ and 0.17, respectively; the mean value of $\sigma_\mu = 2.06 \text{ mm}^{-1}$ is comparable to other tumorous tissues, except for Grade III, and the mean value of $\sigma_K = 0.16$ is the highest, which also reflects the heterogeneity of this type of brain tumor. Thus, due to strong variance of properties, the distinguishing of Grade IV from intact tissues using OCT analysis seems to be uncertain, which is confirmed by relatively high values of errors shown in Table 3. The number of analyzed samples and OCT scans for Grade IV is the highest within our sample set, which can be another reason for such a large dispersion of points on the panels (c) and (d) of the Fig. 7. Nevertheless, the medians of the corresponding histograms almost match those of white matter and cortex (panels (e)–(h) of the Fig. 7).

Comparing the obtained values of attenuation coefficient of human brain tissues with previously reported results in Refs. [33,34], a good agreement was observed. In Ref. [33], normal white matter features $\mu = 6.2 \pm 0.8 \text{ mm}^{-1}$, glioma of low Grade is characterized by $\mu = 3.8 \pm 1.3 \text{ mm}^{-1}$ (newly diagnosed) and $3.2 \pm 0.5 \text{ mm}^{-1}$ (recurrent), glioma of high Grade is characterized by $\mu = 3.6 \pm 1.6 \text{ mm}^{-1}$ (newly diagnosed) and $4.6 \pm 1.4 \text{ mm}^{-1}$ (recurrent). The measured in our work attenuation coefficients 3.96 $\pm$ 0.98 mm$^{-1}$ of Grade I samples, and 3.66 $\pm$ 0.75 mm$^{-1}$ of Grade II samples demonstrate high similarity with the reported values. Attenuation of white matter is slightly higher 7.56 $\pm$ 0.91 mm$^{-1}$, but it is also slightly lower than the value 8.5[8.2; 9.3] mm$^{-1}$ from Ref. [34]. Our results show the small decrease of data for cortex tissue, which was also observed previously [34]. The higher $\mu$ for Grades III and IV may be explained by their increased heterogeneity and the presence of necrotic debris, which was entirely studied in Ref. [34], particularly, glioblastoma with necrosis was characterized by 6.3[5.4; 6.8] mm$^{-1}$, the necrotic tissue – by 7.5[5.3; 7.7] mm$^{-1}$.

It should be mentioned, to the best of our knowledge, the dispersion within the area, compared with the sizes of small heterogeneities like blood vessels, of tissue attenuation coefficient, as well as normalized peak of reflection intensity were not studied previously. Meanwhile, the connection between OCT reflectance and refractive index is rather natural [52]. However, we can compare the tendency of $K$ and $\sigma_K$ with alternatively measured refractive index $n$ changes from healthy to tumourous tissues [46,54,60]. Refractive index of brain tissue is almost constant and more similar to water [52,54]. Nevertheless, due to different types of cells, their density, and scattering, it slightly increases for malignant tumors, especially for glioblastoma [46]. As it is shown in Table 3, this character is reflected by higher mean $K$ for glioma of Grade IV and the increase of $\sigma_K$.

4. Discussion

The most common diffuse glioma is primary glioblastoma. Among all tumors of the CNS, it takes the third place in the frequency of occurrence, but leads among other primary malignant tumors of this localization, i.e. glioblastoma accounts for 15.1% of all primary brain tumors and 46.1% of primary malignant brain tumors [61]. At the same time, glioblastoma is one of the most deadly tumors among all types of CNS malignancies in humans; the five-year survival for this disease is 5.1% [62]. Glioblastoma with IDH mutation, the so-called secondary glioblastoma, according to the generally accepted point of view, arises from preexisting glial tumors of a lower Grade of malignancy, which undergo relatively long evolution (in comparison with the pathogenesis of primary glioblastoma) with a gradual increase in the malignant potential and transformation into glioblastoma [63]. According to the results of the present study (see Fig. 7), glioblastoma
demonstrates the most dispersed properties, whose distribution almost covers those of intact white matter and cortex, measured at the same conditions. Possibly, it could be explained by the complex and variable content of this type of brain tumor, which often includes small and large areas of necrosis [34]. However, from the obtained results, several particular measured points can be differentiated from the intact ones, especially, from white matter, but the errors are rather high.

Although the glioma model 101.8 has the morphological picture and biological properties similar to the human multiform glioblastoma, the evident differences between the results for these two types of brain tissues can be noticed (Figs. 3 and 7). Despite the application of tumor models in animals, particularly rodents, for the development and approbation of intraoperative diagnostic methods is a common approach [55,64,65], recently, it was shown that there are significant differences between homologous types of human and mouse cells, including noticeable changes in the proportions of various cell types, their distribution over the layers in the cortex, gene expression and morphology, which emphasizes the presence of important differences between rodent and human brains [66]. The studies from Refs. [67,68] also confirm the differences in the molecular properties of brain tissue between rodents and humans in the transcriptome, proteome, and functional aspects, as well as differences of transcriptomic and proteomic profiles of human and rodent glioma models [69]. Thus, one should carefully apply tumor models in rodent brains and, in particular, take into consideration the differences that could occur during OCT measurements.

The analysis of the attenuation properties of different Grades reveals that the median value of \( \mu \) increases from low to high Grades approaching the values of \( \mu \) for cortex and white matter, i.e., tumorous tissues of Grades III-IV and intact tissues attenuate optical signal stronger than Grades I-II. Therefore, it opens the opportunities for using OCT for the intraoperative detection of low-grade tumor margins in addition to the fluorescent-based methods [20,23]. At the same time, \( K \) undergoes weak changes between different Grades. Thus, the application of refractive index and its derivatives demonstrates lower efficiency for the differentiation of tissues using OCT with the 1300 nm central wavelength. However, assuming OCT and other modalities for tissue analysis in the adjacent and far spectral ranges, such as terahertz range [70], refractive index is a rather promising physical feature for the differentiation of malignant and intact brain tissues [46].

The heterogeneity of human brain glioma tissues was analysed by estimation of the Full Width at Half-Maximum (FWHM) of the 4D-spatial distribution of parameters \([\mu, \sigma_\mu, K, \sigma_K]\) for each tissue type. For this purpose, within the particular sample set, the central point with coordinates \( P_0 = (\mu, \sigma_\mu, K, \sigma_K) \) and the distance

\[
||P_i - P_0||_e = \sqrt{(\mu_i - \mu)^2 + (\sigma_\mu_i - \sigma_\mu)^2 + (K_i - K)^2 + (\sigma_K_i - \sigma_K)^2},
\]

between \( P_0 \) and each point \( P_i \) from the sample set should be determined. The distribution of distances \( ||P_i,1, N_p - P_0||_e \), where \( N_p \) is the number of points within the array, helped to estimate the FWHM of the considered tissue type (Fig. 8(d)). This parameter reflects the small- and large-scale heterogeneity of tissue structure and optical properties. It demonstrates the growing trend except for the glioma Grade I, which could be caused by the benign character of this tumor type.

Indeed, the tumorous tissues are characterized with different heterogeneity. As it could be seen from Fig. 8(a), in case of a typical histological image of glioma Grade II, there is a moderate and slight increase in the density of the cell layer in some areas, as well as almost no expressed cellular and nuclear atypia; the relative monomorphism of nuclei and cells (a low degree of morphological heterogeneity) is determined, and a slight increase in the intensity of staining of nuclear chromatin is detected. Compared with the previous example, for an anaplastic astrocytoma of Grade III (Fig. 8(b)), a significant increase in the density of the cell layer is detected, as well as nuclear and cellular atypia, which is based on the appearance of a moderately expressed cell and nuclear
polymorphism with the appearance of both small cells with rounded hyperchromic nuclei and large cells with enlarged irregularly shaped nuclei (an increase in the degree of morphological heterogeneity) and heterogeneous distribution of chromatin with an increase in the intensity of its staining in general. In the histological image of anaplastic astrocytoma of Grade IV (Fig. 8(c)), the further increase in the density of the cell layer is revealed, as well as an increase in cell and nuclear atypia with the appearance of cells with hyperchromic ugly and rugged nuclei with an uneven chromatin distribution, the degree of polymorphism of cells and nuclei reaches high values (a high degree of morphological heterogeneity). In addition, there is the presence of necrosis in the tissue. The described examples completely confirm the dependance of FWHM parameter on the malignancy Grade.

Summarizing the obtained results, one can observe the high dispersion of tumorous tissue properties, that can be estimated by OCT. Along with the measurement conditions, sample preparation, and applied OCT system, the tissue properties significantly impact on the analysed signal parameters. Despite the particular OCT measurements of one or several distinct samples can possibly provide the distinguishing between glioma and intact tissue, the increase in the sample set enhances the variability of data and reduces the observed difference. Thus, the application of OCT for the intraoperative diagnosis of human brain tumors is still rather difficult, but it could be combined with other modalities and methods, for example with terahertz spectroscopy, confocal microscopy and visual imaging [46,71–74], for enhancement of diagnosis sensitivity and specificity. One of the promising direction for improvement of OCT approach in neurosurgery is its combination with optical clearing approach [75]; it yields not only the increased penetration depth of brain tissue, but provides information about diffusion parameters of tissue as well, which might be useful for more stable differentiation of tumorous and intact tissues. Nevertheless, OCT is a valuable non-invasive label-free tool for study the brain tumors and their peculiarities, that would be definitely used for the future improvements of neurosurgery diagnosis and the accuracy of tumor margins detection.

5. Conclusion

In the present work, the OCT signals of different ex vivo samples of human and rat brain tissues were studied. From the analysis of OCT images, using a single-scattering approximation, the values and standard deviations of the attenuation coefficient and the normalized peak of reflection intensity were estimated. These four parameters enable the comparison of the attenuation and
dielectric properties of different tissues, as well as their small- and large-scale heterogeneity using the statistical analysis. The essential differences of the analysed parameters for rat and human glioblastoma tissues were observed. Moreover, for human malignant brain tissues, especially of high Grades, a rather strong variance of the mentioned parameters was obtained, which complicates the application of OCT for the intraoperative detection of brain tumor margins. Oppositely, differentiation errors in case of low-Grade gliomas are relatively small, which opens the perspectives for improving the low performance of intraoperative fluorescent-based methods for Grades I and II. Finally, this study showed the growing trend of FWHM parameter, estimated for the distributions of parameters for glioma tissues from low to high Grades, which matches with the increased morphological heterogeneity of these tissues. The results of this research reveal the advantages and weaknesses of OCT for neurosurgical diagnosis and highlight the directions of its further improvements. This study was performed by using OCT system developed for clinical applications and equipped with an endoscope for measuring hardly-accessible tissues and internal organs. Therefore, considering the limited resolution, sensitivity, and scanning range of such systems, the results of this work are of high practical importance for real-life applications.

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**Disclosures**
The authors declare no conflict of interest.

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