Optical Properties of Perfused Rat Liver Tissues

Ksenia Kandurova, Alexander Palalov, Evgeniya Seryogina, Viktor Dremin, Andrey Dunaev, and Elena Potapova

Research and Development Center of Biomedical Photonics, Orel State University, 95 Komsomolskaya str., Orel 302026, Russia

*** e-mail:** kandkseniya@gmail.com

Abstract. In this work, we demonstrate the results of measuring the optical properties of rat liver tissue slices after the liver itself underwent the perfusion procedure with an isotonic solution. The approach is suggested as a means to attenuate the influence of blood absorption on recorded characteristics and demonstrates its effectiveness in changing the composition of recorded spectra in the visible range. The data obtained seem promising to be used to upgrade the proposed methodology and apply the results for the diagnosis and modeling of light-tissue interaction in liver under healthy and pathological conditions.
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Keywords: liver; perfusion; optical properties; spectrophotometry.

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1 Introduction

Optical diagnostic methods remain a promising area of biotechnical research and continue to improve to find new applications in medicine [1–3], including surgery [4–8]. In turn, surgery tends to widely introduce less radical surgical procedures. This trend requires the development of technologies for an objective assessment of the functional state of organ tissues right in the course of surgery. A wide range of continuously evolving spectroscopy and imaging techniques based on various phenomena of light-tissue interaction can be used to obtain real-time diagnostic information [9, 10]. Research aimed at combining new optical methods with standard surgical equipment is of special relevance at present [11–14].

Developing and calibrating new optical diagnostic methods requires knowledge of tissue optical properties (absorption and scattering coefficients, anisotropy factor, refractive index, etc.). To date, many data have been accumulated on the optical characteristics of various tissues [15, 16] (e.g. skin and muscles [17, 18], liver [19, 20], pancreas [21], bones [22], blood [23], brain [24]), including pathological ones [25, 26].

A number of clinical studies in hepatology use liver optical properties to develop approaches for optimizing the treatment [27, 28] or for differentiating between healthy and tumor tissues [4, 29]. However, the fact that the liver is an organ with a high blood supply is not taken into account [20], and therefore it would be important to exclude or reduce the excessive influence of one of the strongest optical absorbers, blood, in some regions of the optical spectrum. Hemoglobin and its derivatives are chromophores that significantly absorb optical radiation in the visible range, affecting the results of measurements by various methods, including those based on excitation and registration of endogenous fluorescence spectra [30, 31].

To reduce the influence of the strong absorption properties of blood hemoglobin on the measured optical characteristics of the liver, it is possible to use a perfusion procedure to remove blood from the blood vessels of the liver using, for example, an isotonic solution (0.9% NaCl).

Organ perfusion is used in biomedical research for several purposes: as a model to study the effects of ischemia and reperfusion in transplantology [32], for preliminary preparation of the liver parenchyma before hepatocyte isolation [33–35], and for obtaining biomatrixes in tissue engineering [36].

Thus, the aim of this work was to measure the optical characteristics of the rat liver using the approach to reduce blood volume by perfusing the organ before preparing the acute slices used for spectrophotometric measurements.

2 Methods and Materials

The studies were approved by the Ethics Committee of Orel State University named after I. S. Turgenev (protocol No. 188 of 21.02.2020) and were carried out in accordance with the principles of Good Laboratory Practice [37].

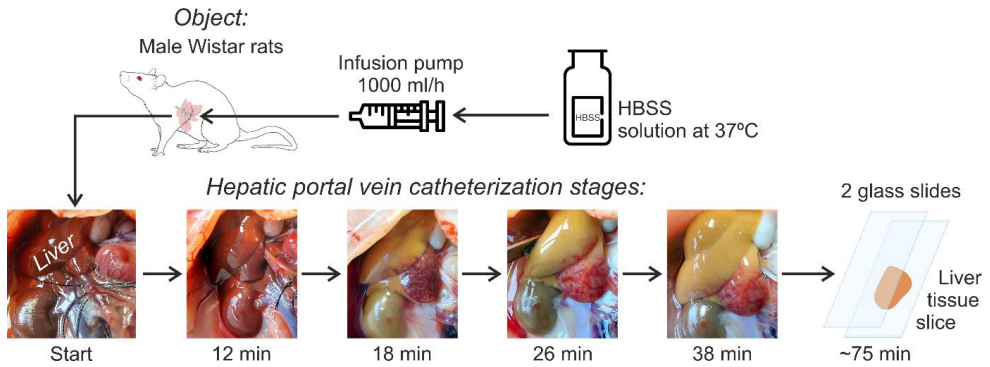


Fig. 1 Schematic of the rat liver perfusion procedure used to prepare tissue samples for optical measurements with an approximate time frame for observations of changes in the liver tissue.

In the study, male Wistar rats (n = 3 per experimental group and n = 3 per control group, age – 3 months, weight – 220–250 g) were used. The experimental studies included two stages: liver perfusion in laboratory animals to obtain liver tissue samples and following spectrophotometric measurements. To assess the efficiency of the perfusion procedure, the optical characteristics obtained were compared with those of the control group of rats, which were measured in fresh slices.

The perfusion procedure was performed under anesthesia. Zoletil 100 (Vibrac, France) and Xyla (Interchemie, The Netherlands) in standard doses were used to anesthetize the animal. The rat was fixed on a special platform in the supine position with a gauze holder. After the onset of deep anesthesia, a layer-by-layer dissection of the anterior abdominal wall along the median line was performed to provide access to the abdominal cavity. The portal vein of the liver was then isolated from the connective tissue space using soft forceps and isolated from the rest of the tissues. Ligatures were placed under the portal vein, one of them prevented blood flow. Subsequently, a vascular catheter (22 G) pre-filled with heparin solution (5000 i.u./ml) was inserted into the vein via a guide. The catheter was fixated with the remaining ligatures and connected by flexible tubes to an infusion system. Hank's Balanced Salt Solution (HBSS), pH=7.4, 37 ℃, was injected into the vessel. The inferior vena cava was perforated to ensure fluid outflow. The flow rate of the solution was 1000 ml/h, the total volume of the solution was about 1.5–2.0 l. The important part of the procedure was to keep the rat's heart working, which accelerated the withdrawal of blood from the liver vessels. In some cases, rapid liver perfusion was performed to eliminate probable stasis in small vessels.

During perfusion (Fig. 1), there was a change in the color of the liver from red-brown to yellow-gray and the vascular pattern became visible on the surface of the organ. The degree of perfusion sufficient for optical measurements was determined by the color of the liver and the intensity of HBSS solution staining with blood washed out. At the end of the procedure, the catheter was carefully disconnected and the animal was removed from the experiment by euthanasia. The liver was isolated from the abdominal cavity and placed in a Petri dish with HBSS solution. The liver slices were made by hand and their thickness (0.9–1.0 mm) was calculated by taking into account the thickness of the glass slides measured by a micrometer MK 0–25 (measuring range 0–25 mm, measuring error 4 μm).

The obtained tissue slices (N = 8 samples in the study group and N = 11 in the control group) were placed between two standard 1-mm silicate glass slides. A fixed distance of 0.95 mm between the glasses was maintained. A Shimadzu UV-2600 dual-beam spectrophotometer (Shimadzu Corporation, Japan) with an ISR-2600Plus integrating sphere module was used to measure the characteristics of tissue samples. The sphere has a diameter of 60 mm with two detectors (photomultiplier and InGaAs detector) and allows recording spectra in the range of 220–1400 nm with a step of 1 nm. The arrangement of the samples relative to the sphere ports (S side – sample side and R side – reference side) is shown in Fig. 2.

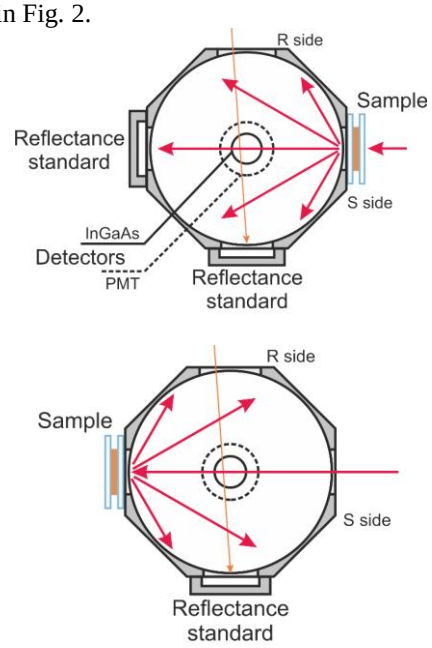


Fig. 2 Schematic of measurements of the optical characteristics of liver tissue samples using an integrating sphere with a double-beam system: (a) total transmittance T_t, (b) total reflectance R_t.

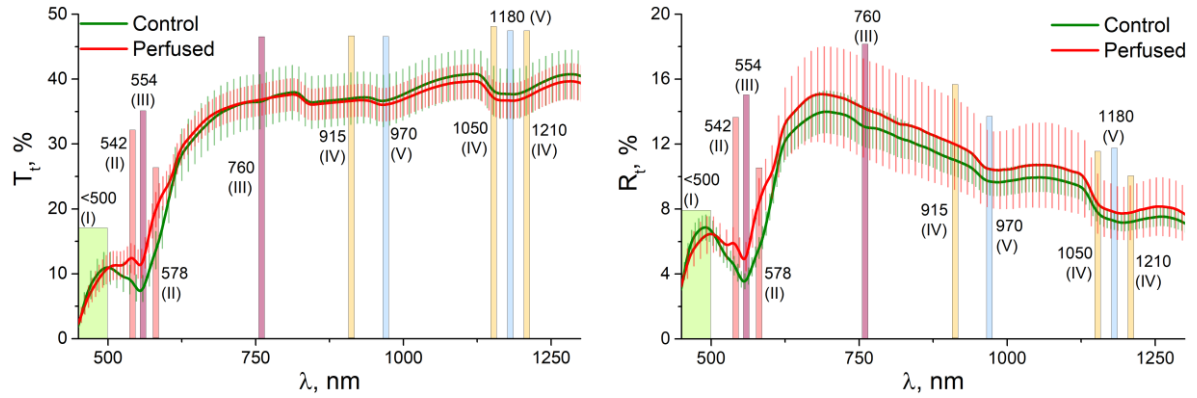


Fig. 3 Average (mean ± standard deviation) spectra of total transmittance T_t (a) and total reflection R_t (b) of rat liver tissue samples subjected (red line) and not subjected (green line) to the perfusion procedure along with typical wavelengths of light absorption by the main chromophores.

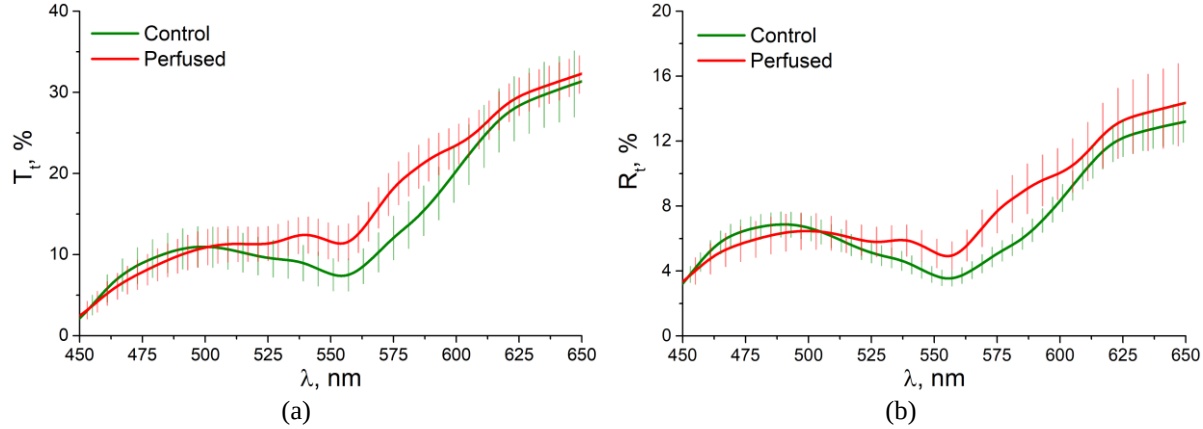


Fig. 4 Average (mean ± standard deviation) spectra of total transmittance T_t (a) and total reflection R_t (b) of rat liver tissue samples subjected (red line) and not subjected (green line) to the perfusion procedure: comparison of results in the 450–650 nm range.

The absorption coefficient μ_a and the reduced scattering coefficient μ_s' were calculated by the inverse adding-doubling method using the algorithm developed and described by S. Prahl [38]. For calculations, the value of the anisotropy factor g was assumed to be 0.7 according to data from the literature for liver tissue [4, 20, 39]. The algorithm used considers the interaction of radiation with the sample sandwiched between the slides. Thus, the calculation takes into account the specular reflectance from the air, glass and tissue borders.

3 Results and Discussion

The results of the spectral measurements obtained for all samples were averaged. Fig. 3 shows the mean and standard deviation of the total transmittance and reflectance values of the obtained rat liver tissue samples.

The spectra show an increase in total transmittance and reflectance with increasing wavelength, as well as a slight decrease in the 500–600 nm range. Regarding the visible range, the observed picture is mainly due to the presence of bilirubin pigment in the tissue (spectral range with the characteristic wavelength of light absorption is marked in Fig. 3 by number I (hereinafter the same for other chromophores)) – one of the main components of bile produced by hepatocytes [40–42] – as well as two forms of blood protein hemoglobin – oxygen-bound oxyhemoglobin (II) and unbound deoxyhemoglobin (III) [23, 40, 43]. In the near-infrared (NIR) region, the spectral profile of liver tissue is largely determined by the intersection of the lipid (IV) and water (V) absorption bands [43–45].

Highly oxygenated blood has two distinctive peaks in the absorption spectrum at 415 nm (Soret band) and at 542 and 578 nm (Q-bands); deoxygenated blood has these peaks at 425, 554 and 760 nm [46]. In the 500–600 nm range, all recorded spectra had a similar characteristic decrease in reflectance and transmittance due to the presence of hemoglobin. The analysis of the data obtained (Fig. 4) revealed that the liver slices of the control group had a typical absorption peak only at a

wavelength of 554 nm. There was also a characteristic decrease in transmittance and reflectance in the deoxyhemoglobin absorption band at 760 nm. The specific characteristic of the liver blood supply is the increase in venous blood content [47], which causes a higher intensity of light absorption by deoxyhemoglobin.

After perfusion was performed, the oxyhemoglobin absorption became dominant in liver slices, though there was a 15–20 nm blue-shift of this part of the spectrum. This can be caused by the influence of the spectral characteristics of bile, which has local absorption maxima at wavelengths of 409 and 605 nm. At the same time, the second absorption maximum of bile becomes well manifested in the spectrum of perfused liver tissue (Fig. 4).

Therefore, despite the performed perfusion and the visual control of the liver parenchyma during the procedure, a portion of the blood remained in the small vessels, but had a noticeably smaller effect on the results of the spectral characteristics study.

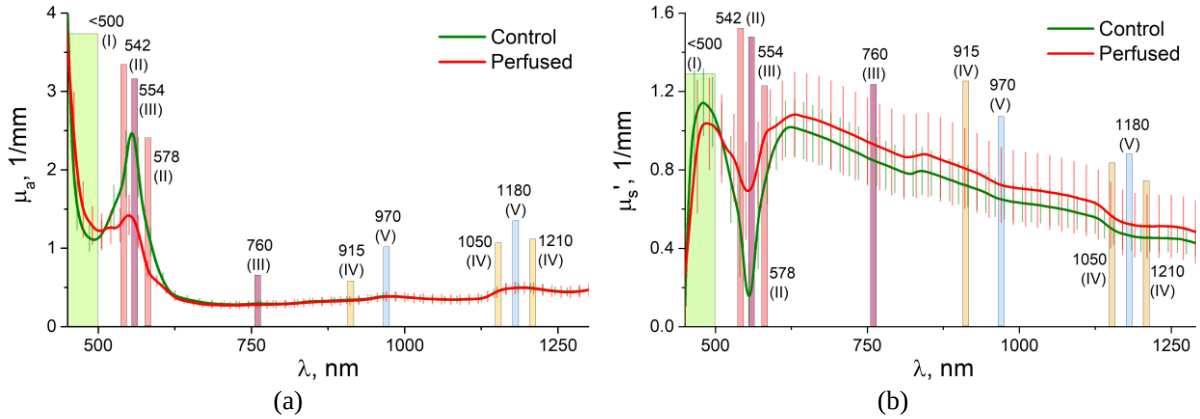
After perfusion, the total transmittance values slightly decreased in the NIR range, while the total reflectance increased. There were no changes in the shape of the spectra, which may indicate quantitative rather than qualitative changes that were observed in the oxy- and deoxyhemoglobin absorption ranges. The HBSS solution is aqueous, which played a role in these studies when it was used to displace blood in liver vessels. As stated above, the NIR range is informative for assessing changes in the water and lipid content. According to the presented results, there is a characteristic decrease at wavelengths around 970 and 1180–1190 nm in the perfused tissues due to water absorption [44, 48, 49].

Fig. 5 shows graphs of the calculated dependences of the absorption and scattering coefficients as a function of wavelength. The absorption coefficient showed greater reproducibility (less standard deviation) compared to the scattering coefficient and the expected ratios of values in the bands of different main chromophores [4, 20, 49, 50]. The values remained almost unchanged in the NIR range, while there was a twofold decrease at the band of 540–560 nm as a result of the perfusion procedure.

The results of the calculation of the reduced scattering coefficient dependence clearly demonstrate the intended effect of the proposed approach for wavelengths below 600 nm. Due to the high blood content and, therefore, the low values of total transmittance and reflectance within the hemoglobin absorption bands, the calculated scattering coefficient has critically low values, which are not observed after liver perfusion. In general, the dependence of the reduced scattering coefficient was as expected – it decreased when the wavelength increased [19, 20, 39].

The following limitations of the experimental study should be noted. Control of the perfusion level was provided only by visual inspection. Despite attempts to standardize the procedure (minimum volume of pumped liquid, perfusion rate, etc.), its result was determined, among other things, by individual peculiarities of the object (anatomy and viability of the rat). The liver slices were taken by the same lab technician, but manually according to a specially developed technique, which affects their thickness and area. Furthermore, the irregular structure of the biological tissue and the methodological difficulties of fixing the slices between the glass slides may have influenced the results of the study.

Thus, the proposed approach for preliminary perfusion of liver tissues to reduce the influence of blood on the optical characteristics of the liver demonstrated its effectiveness, which allowed it to significantly reduce the influence of oxy- and deoxyhemoglobin on spectra recording results.



(a) (b)

Fig. 5 Average dependences (mean ± standard deviation) of calculated absorption μ_a (a) and reduced scattering coefficient μ_s' (b) of rat liver tissue samples subjected (red line) and not subjected (green line) to the perfusion procedure along with typical wavelengths of light absorption by the main chromophores.

4 Conclusion

Currently, an important step in the development of biomedical optics is the transition from theoretical and preclinical research to the practical implementation of technologies in clinical practice. Adequate and effective application of optical methods in diagnostics, surgery and therapy requires reliable data on the optical properties of human tissues, including the liver. In this work, we considered, in particular, acquisition of optical properties of liver tissue along with the decreasing influence of optical characteristics of blood. This can be of importance when modeling spectral characteristics of the liver in diseases such as steatosis, cirrhosis, cancer, etc. The results presented in the work have shown that the preliminary perfusion of the liver allows one to reduce the influence of hemoglobin on the recorded and calculated optical parameters.

Disclosures

The authors declare no conflict of interest.

Acknowledgements

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