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The application of the multimodal approach for studying optical properties of bile in obstructive jaundice

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ABSTRACT

This article describes the results of experimental studies using optical methods for *in vitro* estimation of the composition of bile obtained from patients with different etiologies of obstructive jaundice. Experimental studies were carried out using spectrophotometry and Raman spectroscopy methods to study and compare optical properties of bile from patients with obstructive jaundice caused either by choledocholithiasis or malignant tumors. The results show that the selected methods are suitable for studying the composition and optical properties of bile and can provide additional diagnostic information. Averaged Raman spectra, as well as absorption spectra of bile, corresponding to different etiologies of jaundice origin were demonstrated.

Keywords: obstructive jaundice, liver, bile, spectrophotometry, Raman spectroscopy.

1. INTRODUCTION

Diagnostics and treatment of hepatopancreatoduodenal diseases complicated by biliary obstruction remain a relevant medical problem. Obstructive jaundice is a syndrome that develops when the outflow of bile through the intra- and extrahepatic biliary tracts into the duodenum is disturbed. Biliary obstruction can be caused by cholelithiasis, inflammatory processes, as well as benign and malignant diseases of the hepatopancreatoduodenal organs. Bile duct obstruction causes hepatocyte dysfunction and leads to impaired detoxification and synthetic functions of the liver. As a consequence, severe hemodynamic, metabolic, coagulation and immune changes develop, leading to functional and morphological disorders of the liver and other vital organs[1]. Every year the number of patients with obstructive jaundice of various etiologies increases. This trend occurs mainly due to changes in lifestyle and diet.

Despite the high level of modern research methods, differential diagnosis of obstructive jaundice causes remains a difficult task due to the patient's condition requiring urgent medical intervention. A surgeon is significantly limited in the means and time for identification of the underlying disease, so the biliary decompression becomes a primary task[2]. Improvement of differential diagnosis and timely determination and prognosis of such a serious complication as liver failure is of great importance for reaching better treatment results[3]. A clear understanding of the structural and functional changes occurring in the liver is the key to the success of further therapy, consisting of the combination of conservative and surgical treatment. However, there are no informative criteria for evaluation of the structural and functional state of the liver in this pathology, as well as a rather simple and fast technology for this evaluation. Therefore, there is no consensus on the role, place and sequence of performing diagnostic and treatment procedures for diseases of the hepatopancreatoduodenal organs complicated by obstructive jaundice.

Currently, biophotonics methods are still finding new applications in various fields of medicine and biology[4–6]. One of perspective directions of optical diagnostics is development of scientific and instrumental bases for *in vivo* analysis of functional state of liver[7,8] as well as *in vitro* estimation of bile composition[9]. It is proposed to use a multimodal approach to the study of bile using different methods of optical diagnosis to metabolic changes in the liver through bile composition.

Measurement of *ex vivo* and *in vivo* optical properties of biological tissues and substances was made possible by the recent developments of optical methods in the areas of diagnostics, therapy and surgery[10–12]. The knowledge of optical properties

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and its possible changes caused by various pathologies can be used for the development and implementation of other optical methods as well as being a valuable and sensitive criterion for clinical applications[11,13]. It is shown that the wide range of optical properties measurements can be carried out using commercially available spectrophotometers.

Another optical method – Raman spectroscopy – is considered to be a promising noninvasive monitoring approach[14,15]. The phenomenon of Raman scattering is observed when monochromatic radiation is incident upon optically transparent media. The difficulty that arises when studying samples by this method is the strong presence of a fluorescence signal in the raw spectra. The solution is to use existing and to develop new algorithms to extract the fluorescence spectrum of a sample from the total registered spectrum in order to distinguish Raman peaks. Raman spectroscopy is widely used to study the composition of biological fluids[16–18].

The article presents the results of the *in vitro* stage of pilot studies of bile samples from patients with different etiologies of obstructive jaundice using the multimodal approach, which includes spectrophotometric measurements and Raman spectroscopy.

2. MATERIALS AND METHODS

The study was approved by the Ethics Committee of Orel State University (record of the meeting No. 14 of 24.01.2019). Bile samples from 3 patients with choledocholithiasis and 3 patients with malignant tumors were obtained during antegrade decompression of the biliary tract under ultrasound and X-ray control. The studies were carried out the same day no later than 5 hours after obtaining. The experimental procedure involved the use of the following methods and equipment (Fig. 1).

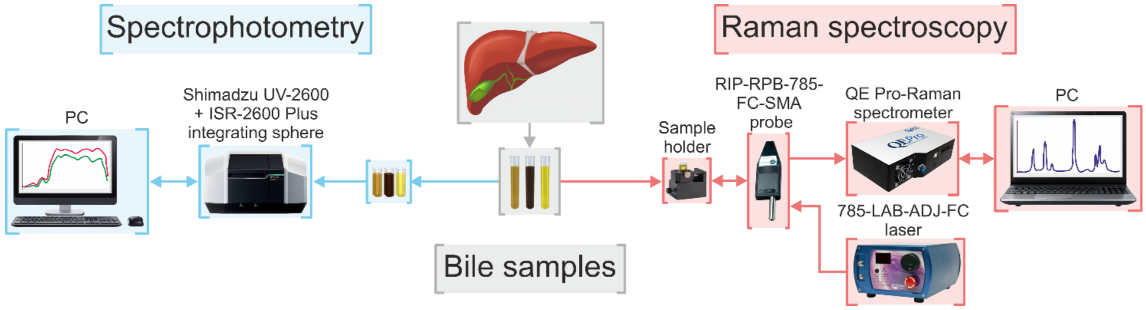


Figure 1. Schematic view of the proposed methodology.

Spectral characteristics (total transmittance and total reflectance) of bile samples in 1 mm quartz cells were measured using a Shimadzu UV-2600 (Shimadzu Corporation, Japan) spectrophotometer with an ISR-2600Plus integrating sphere in the range of 220-1400 nm.

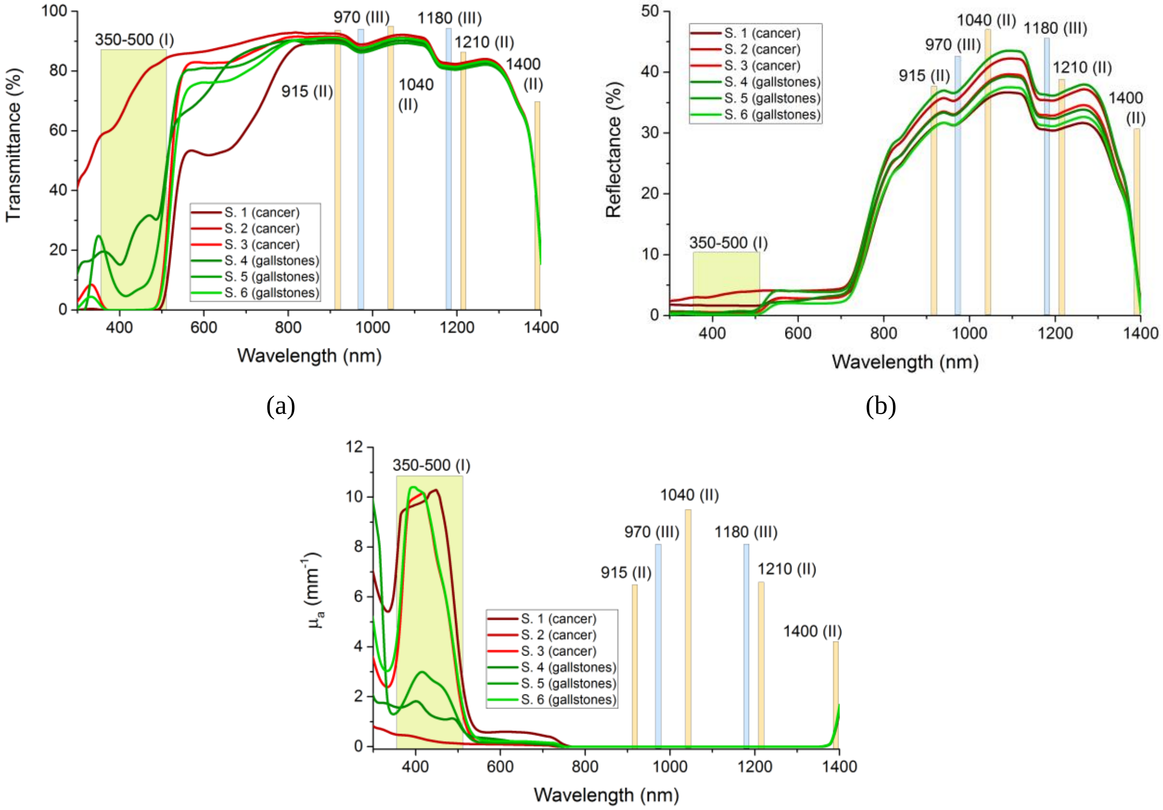
Raman spectra were recorded using a QEPRO-RAMAN (Ocean Optics, USA) spectrometer in the range of 150 cm^{-1} - 2100 cm^{-1}. 785 nm laser 785-LAB-ADJ-FC (Ocean Optics, USA) was used to excite the radiation and a fiber-optic Raman probe RIP-RPB-785-FC-SMA (Ocean Optics, USA) with a focal length of 7.5 mm was used to deliver the exiting radiation, as well as to collect and filter the scattered radiation. The laser power at the focal distance was 30 mW, the exposure time was 20 s. Liquid bile samples containing natural precipitated admixtures were studied without preliminary mixing. The bile was poured into 10 mm quartz cells and placed in a Raman sample holder connected to the probe.

Three spectra were recorded and averaged in each sample. To obtain representative Raman spectra of bile in different pathologies, the previously obtained spectra were also averaged among three samples of the same etiology.

3. RESULTS AND DISCUSSION

The obtained results demonstrate the differences between the pathologies in spectral composition and amplitudes of the parameters recorded by both optical methods.

The results of spectrophotometry measurements are demonstrated in Figure 2 a,b. The data were used to calculate absorbance coefficient in each sample (Fig. 2 c) using inverse adding-doubling method[19].Total transmittance and reflectance spectra show higher values in the near infrared range and a significant decrease in the visible range. The content of the main component of bile – bilirubin (labeled as I in Fig. 2) – causes higher light absorption coefficient in five out of six examined samples in the range of 350-500 nm[13,20]. Sample 1 of patients with malignant tumor was the most transparent liquid, so the influence of bilirubin on spectra was less observed there. The shape of the spectra in the near infrared range is mostly determined by the content of lipids (labeled as II) and water (labeled as III) in bile (the wavelengths of characteristic absorption bands for these chromophores are specified in Fig. 2)[21,22]. Less significant differences compared to the visible range may indicate that their content in the studied samples remains more constant.



(a)

(b)

(c)

Figure 2. Typical spectra of cancer (red lines) and gallstones (green lines) bile samples properties: total transmittance (a), total reflectance (b), and absorbance coefficient (c).

Raman spectroscopy data were obtained for all bile samples examined. Averaged spectra recorded in bile samples collected from patients with choledocholithiasis and with malignant tumors are shown in Figure 3.

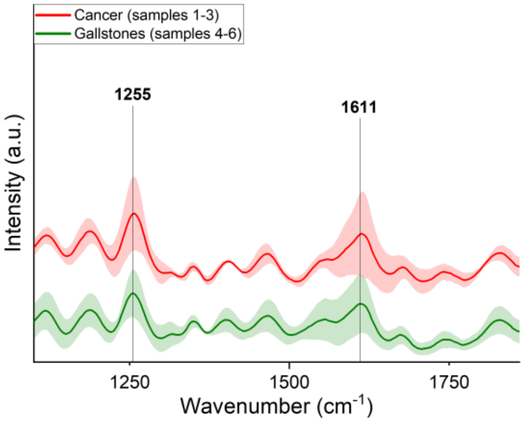


Figure 3. Typical averaged (mean ± standard deviation) Raman spectra of cancer (red line) and gallstones (green line) samples.

The fluorescent background was subtracted from the recorded spectra using Origin software (OriginLab Corporation). The baseline anchor points have been found by the second derivative method.

Typical constituents of bile include water, solids, biliary acids, bile pigments, cholesterol, lipids and fatty acids[23,24]. However, the data obtained in this research are preliminary and does not reflect the full chemical composition of bile. The methodology originally chosen for implementation does not allow extracting the Raman peaks in the 450 to 1100 cm^{-1} region because of the low signal-to-noise ratio. As for ways to improve the quality of data obtained by Raman spectroscopy in the future, we can highlight the use of special SERS substrates, increasing exposure time or laser radiation power. As the examination of the samples occurs *in vitro*, the limiting factor in determining the optimal exposure time and radiation power will be the point at which a sample would be photodestructed.

At this stage of research, the task was to identify the major vibrational bands in the remaining region from 1100 to 1860 cm^{-1}. To better understand the molecular basis for the observed Raman spectra, Table 1 lists the wavenumbers and tentative assignments according to the data from the literature[25,26]As bilirubin is one of the main components of bile, it is expected to be present in spectra of all the samples. The wavenumbers correspond to vibrational modes of biomolecules.

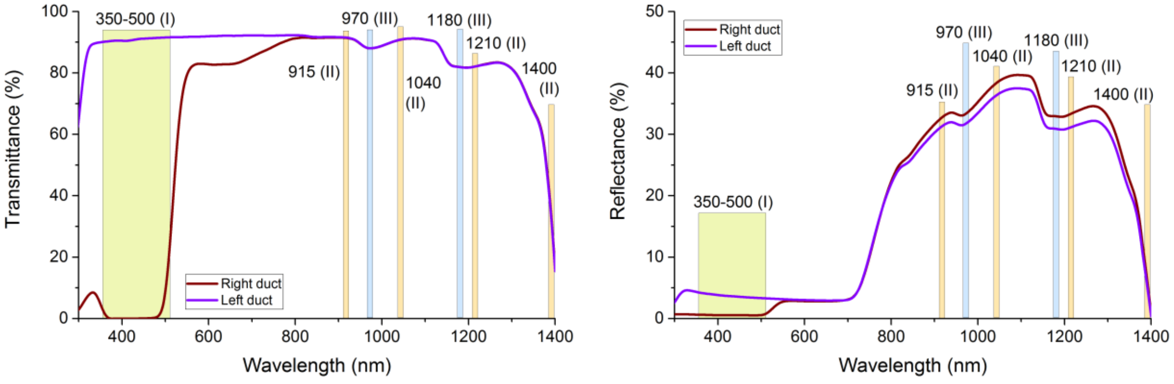
Table 1. Peak positions and tentative assignments of major vibrational bands observed in Raman experiments.

Peak position (cm^{-1})	**Assignment**	**Reference**
1255-1260	Lactam ring C-C stretching and N-H bending (bilirubin)	[25, 26]
1606-1616	C-C stretching and N-H bending (bilirubin)	[25, 26]

As a result, two major bands centered at 1611 cm^{-1} and 1255 cm^{-1} were observed. Thus, the allocated Raman peaks of the samples could be largely originated from bilirubin and bilirubin derivatives. The Raman spectroscopy data we acquired are similar to those obtained by T.D. Vu et al in their voltage-applied SERS measurements of bile juice[27].

It is worth noting that one of the cases was considered separately. One of the patients had a significant difference in the color of bile obtained from the separated biliary systems of the right and left lobes of the liver. Due to the presence of a tumor blocking the biliary tract in portal fissure, bile was taken separately from right and left bile ducts.

The optical properties data obtained with spectrophotometry are demonstrated in Figures 4 and 5. On visual inspection, the bile of the right duct had a characteristic brown-green color, while the fluid of the left duct was not colored and is almost transparent. This difference is observed in all three plots in the visible range. At the same time, when shifting to the near-infrared region, the changes become insignificant or absent at all.



(a) (b)

Figure 4. Typical spectra of bile samples properties from the right duct (dark red line) and the left duct (purple line): total transmittance (a), total reflectance (b).

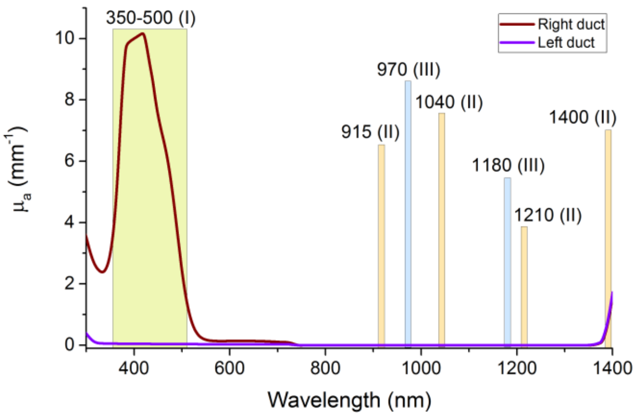


Figure 5. Typical spectra of absorbance coefficients of bile samples from the right duct (dark red line) and the left duct (purple line).

The results of Raman spectroscopy are shown in Figure 6. Despite the shortcomings of the currently used methodology for obtaining Raman spectroscopy data, a significant difference in Raman spectra of bile obtained from different ducts was noted.

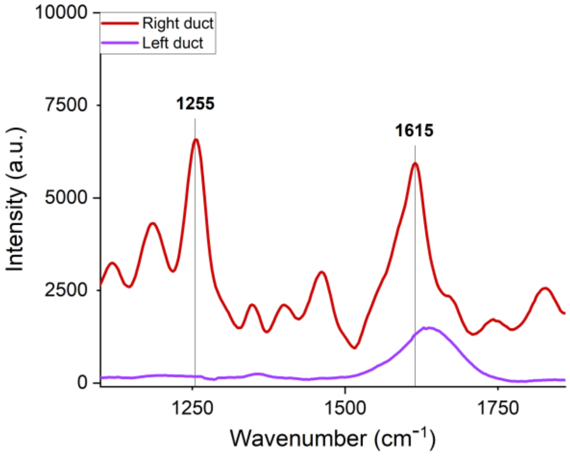


Figure 6. Typical Raman spectra of bile samples from the right duct (dark red line) and the left duct (purple line).

The reason for the differences in the observed spectra may be the different size of the lobes, the volume of ducts in them, and the degree of block for each lobe. The following factors affect the chemical composition of bile produced, including its bilirubin content. Thus, in the presence of pathology, a decrease in the amplitude of bilirubin peaks as well as changes in the shape of the spectrum in general, were detected.

4. CONCLUSION

The described results show the possibility and sensitivity of the proposed methods for the *in vitro* analysis of bile samples from patients with obstructive jaundice of various etiologies. To summarize, we can say that Raman spectra as well as dependences of the total transmittance, reflectance and the absorption coefficient revealed the presence of characteristic bile components. Unfortunately, at this stage it was difficult to determine the clear difference between the etiologies of jaundice.

In order to assess in more detail, the influence of differences between the causes of obstructive jaundice and the degree of liver failure on the chemical composition of bile in the future it is planned to continue the acquisition of experimental data in order to identify informative markers of hepatocyte condition.

Thus, the proposed approach demonstrates promising and broad possibilities both for obtaining new knowledge about the optical properties of bile in different pathologies and for further application of the results in the form of diagnostic criteria for the development of a new multimodal diagnostic technology. Another plan for the next step to supplement the presented protocol is to add in vivo studies in the bile ducts by fluorescence spectroscopy and diffuse reflectance spectroscopy.

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