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DIFFERENT TYPES OF COLORECTAL CARCINOMA CHARACTERIZATION USING STAINED AND NON STAINED PLATES BY OPTO-MAGNETIC SPECTROSCOPY

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Abstract: According to the World Health Organization cancer is the leading cause of death worldwide and on the third place is the most common and dangerous colorectal cancer with the rate of 17.3/100,000 people. Therefore, it is necessary to find effective solutions to improve the quality and speed of diagnostic tools so that they can respond to the challenges of prevention strategies which are an integral part of the rapid diagnosis of a large number of patients. The objectives of the research conducted in this study (*in vitro*) are: (1) examining the possibilities of application of opto-magnetic imaging spectroscopy for characterization between different types of colon cancer, stained and non-stained plates, (2) method validation, (3) parameters determination for research in *in vivo* conditions, and (4) application in clinical trial to increase the efficiency and give more reliable diagnosis. In this paper we present Opto-magnetic imaging spectroscopy (OMIS) as a novel optical method for differentiation of different types of colon cancer, significant similarity between stained and non-stained plates based on light-tissue interaction. Results have showed that OMIS can make a difference between colorectal carcinoma and other types of colon cancer, as well as prove that plates staining can be avoided.

Keywords: colon, stained and non-stained plates, opto-magnetic imaging spectroscopy, screening.

1. INTRODUCTION

According to the World Health Organization (WHO), cancer is the leading cause of death worldwide. In 2008 7.6 million people died of cancer, which is 13% of all deaths. Colorectal cancer is on the third place with a rate of 17.3/100,000, just behind the lung cancer and breast cancer. 1.2 million people are estimated to be annually diagnosed with colorectal cancer (one new every 3.5 minutes) while 609 thousand die (one person every nine minutes) [1].

In the last few years, the rate of deaths from colorectal cancer has decreased in the U.S.; in some highly developed countries it continues to grow (Japan, Finland and Norway), and in others it remains stable (France and Australia). The main reason for reduction in mortality rates was an introduction of screening and removal of precancerous lesions and/or improvement of therapy, while the growth rate can be explained by the transition to the Western way of diet, an increased intake of foods rich in animal fat, high prevalence of obesity, low prevalence of physical activity [2,3].

Serbia belongs to the countries with increased incidence of colorectal cancer. This can be explained by the lack of adequate preventive programs and long waiting for the screening program. From these facts, it can be concluded that colorectal cancer is not brought under control in highly developed countries, that some of these countries even show a growth rate. All this proves a necessity of introducing new methods to improve screening [4].

Therefore, it is necessary to find effective solutions to improve the quality and speed of diagnostic tools so that they can respond to the challenges of prevention strategies which are an integral part of the rapid diagnosis of a large number of patients.

The objectives of research conducted in this study (*in vitro*) are: (1) examining the possibilities of application of opto-magnetic imaging spectroscopy for characterization between different types of colon

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cancer, and to show similarities between stained and non-stained plates, (2) method validation, (3) parameters determination for research in *in vivo* conditions, and (4) application in clinical trial with the aim to raise the efficiency and give more reliable diagnosis.

The main reasons why the so-called "golden standard" – colonoscopy is avoided today are: discomfort, invasiveness and potential adverse effects of the method. Opto-magnetic imaging spectroscopy is a noninvasive method which allows some comfort to the patients. So far, this method was successfully applied in other fields of scientific research [5−9].

2. METHOD:

Opto-Magnetic Imaging Spectroscopy, (OMIS), is a nanophysical diagnostic technique based on interaction of electromagnetic radiation with valence electrons within the sample material, and hence examining electronic properties of matter (covalent bonds, hydrogen bonds, ion-electron interaction, and Van der Waals interaction). Bearing in mind that the orbital velocity of valence electron in atoms is approximately 10^6 m/s, calculation gives the ratio between the magnetic force, (F_M), and electrical force, (F_E) of matter is $F_M/F_E \approx 10^{-4}$. Since force, (F), is directly related to action ($A = F \cdot d \cdot t$, where F represents the force within the range of 0.01 – 1.0 nN, d is displacement within the range 0.1 – 5.0 nm, and t is the time within the range 10^{-8}–$10^{-10} s$), it can be concluded that the magnetic force of matter is by four orders of magnitude closer to quantum state of matter than the electrical force. This opens an opportunity to detect the conformational states and changes in the matter on nanoscale level using light-matter interaction method [10].

OMIS method deals with obtaining paramagnetic/diamagnetic properties of materials/ colon tissue, (unpaired/paired electrons) based on their interaction with visible light. The basic tool is the light of the wavelength in the range between 400 nm and 700 nm. Since the energy of valence electrons and photons of visible light has the same value, imaging by visible light is non-invasive and allows an examination process that can be repeatedly conducted without presenting any risks to the patient or sample material damage.

The light as an electromagnetic phenomenon consists of electromagnetic waves that are perpendicular and can be split under specific conditions, which means that the light can be polarized. One particular type of light polarization occurs during the interaction of light and matter at a specific angle, known as the Brewster's angle. Each type of matter has unique angle value of Brewster's angle. For example, Brewster's angle for water-air interface is 53° (refractive index n = 1.33). When a sample is illuminated under this specific angle, the reflected light will be polarized. Reflected polarized light contains only electrical component, in longitudinal wave, and in transversal wave (perpendicular to longitudinal), magnetic component of light-matter interaction. Since longitudinal wave directly influences CCD/CMOS sensor, while transversal has a negligible influence, subtracting the reflected polarized light (electrical properties) from the reflected white diffuse light (electromagnetic properties) will consequently provide information about magnetic properties of matter based on light-matter interaction.

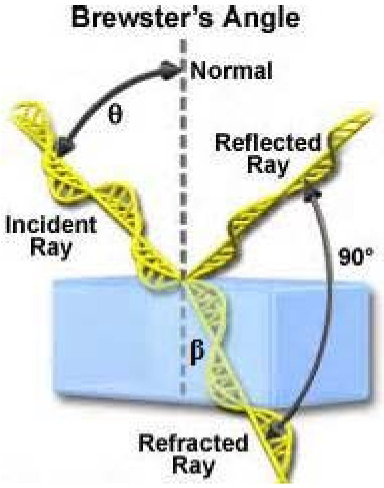

Figure 1. An illustration of the incident, reflected and refracted light rays with respect to the boundary between two media. The incident and the reflected angles are denoted as θ, while β is the angle of refraction. If α is equal to the Brewster angle, than equality θ+β=90° holds.

Digital images acquired using OMIS method are RGGB (red, double green, and blue) images because the camera is adapted to human visual system. The image processing algorithm is based on "Maxwell triangle" chromaticity diagram and allows conversion of digital image to Opto-magnetic spectra through several operations beginning with creating histograms for each color channel and subsequent conversion of histograms to spectra. As a result, for a pair of digital images of the sample acquired under the white diffuse light, and white diffuse light under Brewster's angle – three spectra are created: blue, red and green for each image. When blue, green or red spectra for image of the sample taken under Brewster's angle are subtracted from blue, green or red spectra for image of the sample taken under the white diffuse light, the resultant composite spectrum will represent opto-magnetic spectrum of the sample. This resultant spectrum is presented in a coordinate system where x axis presents the wavelength difference (WD) measured in nanometers and y axis as Intensity in normalized arbitrary units. The most commonly (R-B)&(W-P) type of Opto-magnetic spectra is used for tissue samples since the blue light is reflected from surface (and very/very thin layer) and the red light from deep tissue layers. In this case we avoided the reflection from surface (as natural reflected mirror effect), because we need information about tissue characteristics [10].

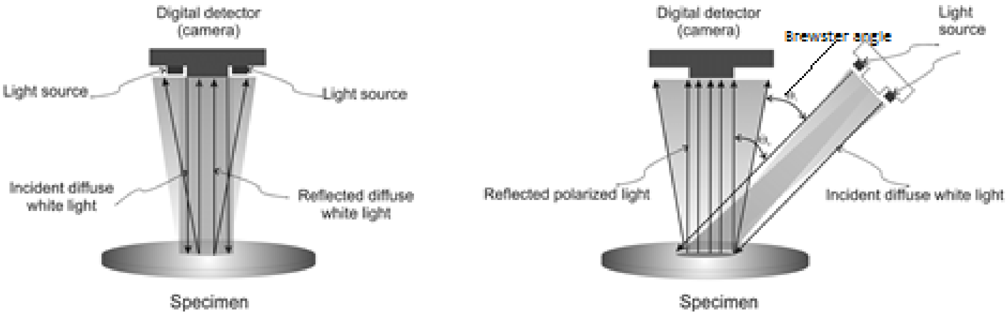

Figure 2. The principle of operation of a device in the opto-magnetic imaging spectroscopy [10]

The basic operational setup for Opto-Magnetic Imaging Spectroscopy (OMIS) consists of a customized housing for Canon digital camera (model IXUS 105, 12.1 MP) with a system of emission diodes at an appropriate angle and a sample holder. The illumination system consists of six LED diodes arranged in a circle (120º) and placed in front of the camera lens it provides illumination of the sample with white diffuse light and white diffuse light under Brewster's angle. The recording can be conducted over area circle, 25 mm in diameter [11].

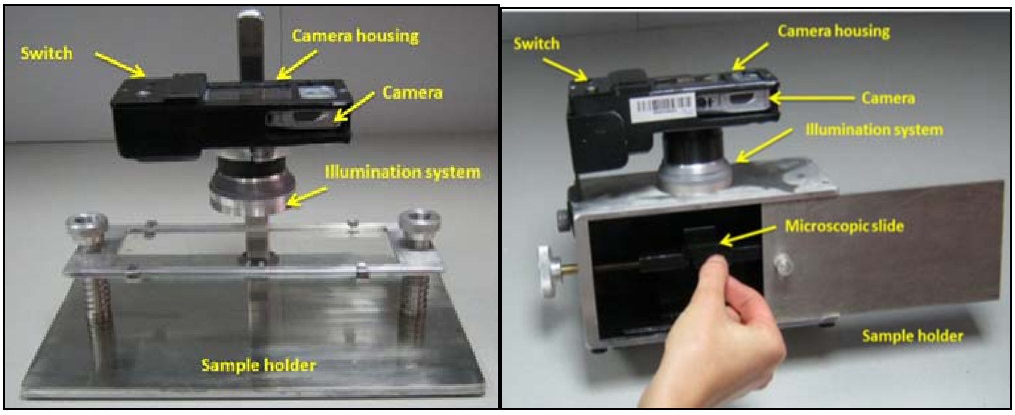

Figure 3. Opto-magnetic imaging spectroscopy system for ex vitro and in vitro applications (NanoLab, Faculty of Mechanical Engineering, University of Belgrade)

The recording procedure comprises the following steps:

1) The illumination of a sample with white diffusive light, 2) acquisition of the first digital picture, 3) illumination of a sample with white diffusive light under Brewster angle, 4) acquisition of the second digital picture.

After recording both digital pictures ten times, which takes approximately 5−10 s per sample, the spectral image processing is performed in three steps:

1) In the first phase, the area of interest is cropped from original picture and all further processing is conducted over that region. After cropping, the region is resolved on component channels (Red – R, Green – G, Blue – B) from which three monochromatic pictures are obtained.

2) In the second phase, the convolution of the spectra in the region of the blue and the red channels is conducted. Next, the difference between the responses of the material sample under white light and polarized light illumination is calculated.

3) In the third phase, the analysis of the spectra is performed by forming the classification of the samples according to the intensities and wavelengths.

Raw pictures obtained in the measurement procedure are cropped in the Adobe Photoshop software to regions with 900x900 pixels resolution. The regions are then processed with spectral convolution code in the MATLAB. The output data are presented in histograms.

3. MATERIAL

Experimental investigations presented in this paper were conducted in The First Surgical Clinic, the School of Medicine, Belgrade University, Serbia as well as at the Department of Biomedical Engineering, Faculty of Mechanical Engineering, Belgrade University, Serbia. Patients of both sexes, aged 19 to 85 years, were included. The tissue samples were received from 50 human patients with histopathology of colon cancer. After surgical resection, each removed colon sample was first rinsed with pure water and then placed on equipment designed for OMIS.

Digital images of healthy tissue and neoplasms were taken 1 hour and 4 hours after tissue removal. Imaging of healthy tissue was at least 8cm from the tumor. After imaging, tissue sample was fixed in formalin for further histopathological examination.

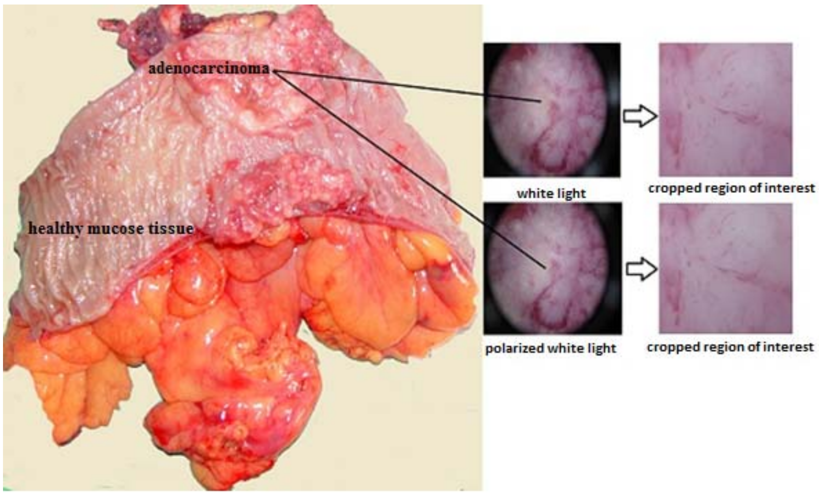

Figure 4. Distant images of cancer tissue (left) and contact image obtained by using OMIS-a (middle) and cropped region of interest (right)

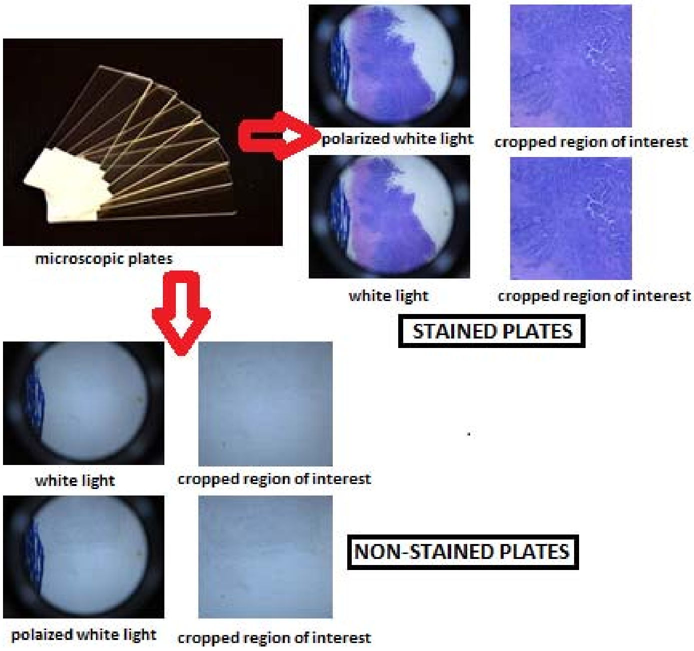

Figure 5. Distant images of histopathological plates, stained and non-stained.

Digital images of histopathological plates were obtained as well, all 20 times. Comparing images difference between the white and polarized white light at stained and non stained plates does not exist, however, image processing by Matlab software package shows a clear difference.

4. RESULTS AND DISCUSION:

Digital images of the observed tissues were processed, and the results are presented with diagrams and tables with typical values of intensity and wavelength differences. In this paper, three representative samples of adenocarcinoma, the most common form of colorectal cancer, and one sample of MALT lymphoma, melanoma and planocellular carcinoma are presented. Research in all cases was carried out in the same conditions in order to reduce the possibility of errors in signal acquisition and data processing.

In Figure 10, adenocarcinoma of the affected tissue, partially overlapping similar behavior of tissue in the paramagnetic and diamagnetic domain was found. Typical values of all three tissues in the domain (R-B) & (W-P) are presented: for Sample 1 − (112,073,25,356), (116,036, −17,754); Sample 2 − (112, 846,23,201), (116,637, −22,294); Sample 3 − (116,423,21,065), (117,993,−20,019). Comparing this diagram of adenocarcinoma with diagrams in Figures 12, 14 and 16, completely different activities in diamagnetic and paramagnetic domain, the intensity and the wavelength difference can be observed. The tissue affected by MALT lymphoma shows the activity in the domain of difference wavelengths of 107− 175nm with peak intensities from −5 (diamagnetic zone) to +7 (paramagnetic zone). In contrast, the tissue affected by melanoma shows the activity in wavelengths difference 119−175 nm and the intensity of −3 − +3 normalized units. The last sample, planocellular carcinoma shows the activity in wavelengths difference from 105−145 nm and the intensity of −4 − +4 normalized units. The results obtained show that the method of opto-magnetic imaging spectroscopy is a sufficiently sensitive method for the classification of tissue and that it clearly shows the difference between the various types of cancer, as well as the similarities with the same species. Further research needs to be done on a bigger number of samples.

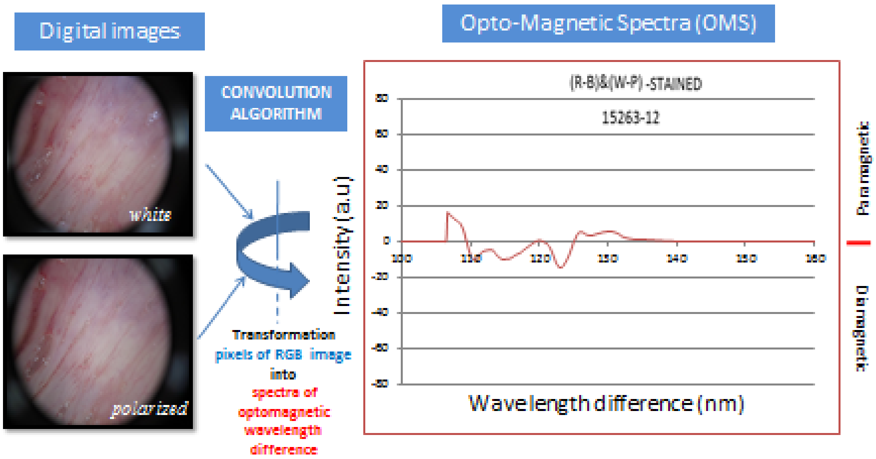

Figure 6. Convolution algorithm of digital image processing and its appropriate diagram

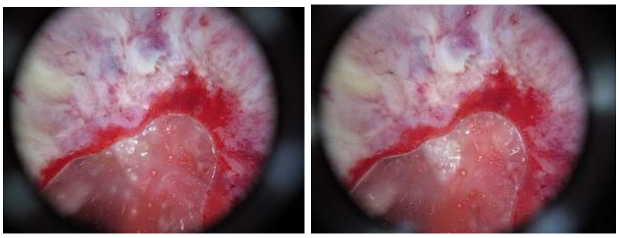

Figure 7. Sample 1 − adenocarcinoma, images obtained by diffuse white light and white reflective polarized light

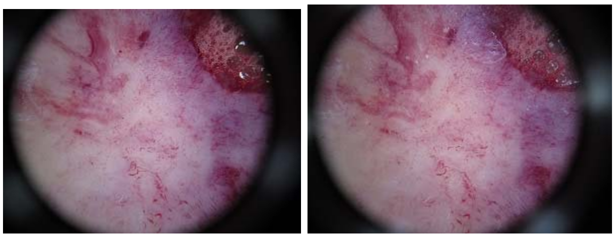

Figure 8. Sample 2 − adenocarcinoma, images obtained by diffuse white light and white reflective polarized light.

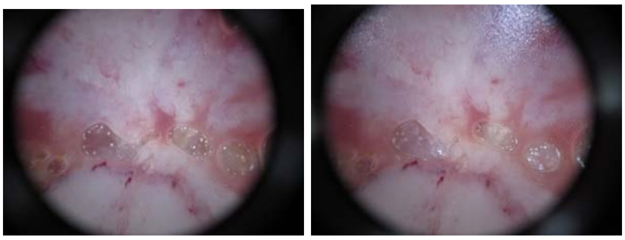

Figure 9. Sample 3 – adenocarcinoma, images obtained by diffuse white light and white reflective polarized light

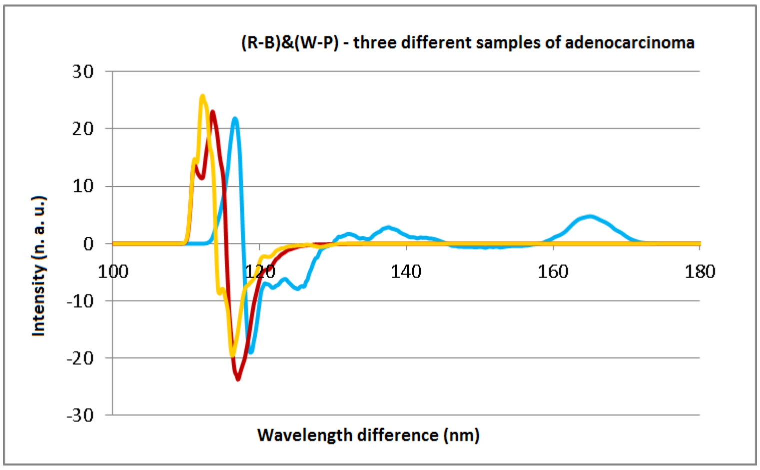

Figure 10. OMIS diagrams for three different adenocarcinoma samples. Typical values for the domain [(RB) & (WP)]: Sample 1 − yellow color (112 073, 25 356), (116,036, −17,754); Sample 2-red (112,846, 23,201), (116,637, −22,294); Sample 3 − blue color (116 423, 21 065) (117 993, −20 019)

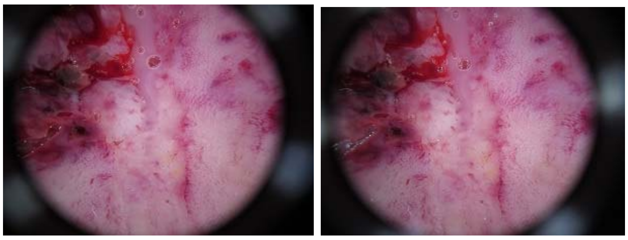

Figure 11. Sample MALT LYMPHOMA, images obtained by diffuse white light and white reflective polarized light

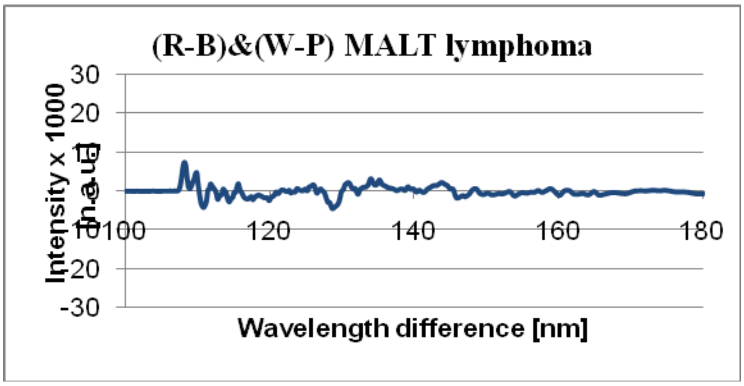

Figure 12. OMIS diagram for Malt lymphoma specie of colon tissue

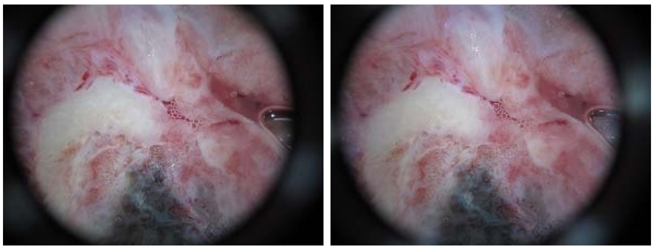

Figure 13. Sample MELANOMA, images obtained by diffuse white light and white reflective polarized light

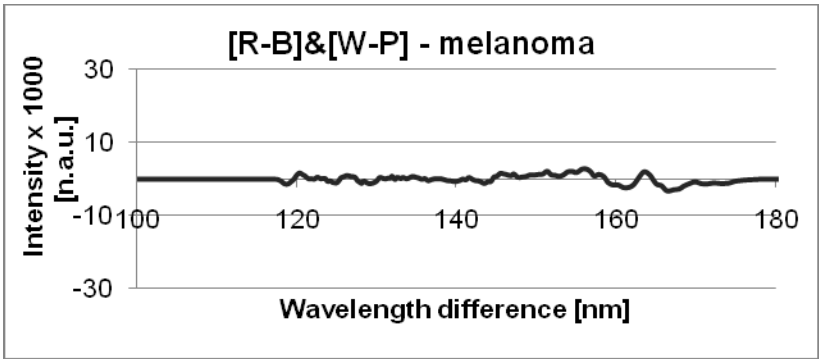

Figure 14. OMIS diagram for melanoma specie of colon tissue

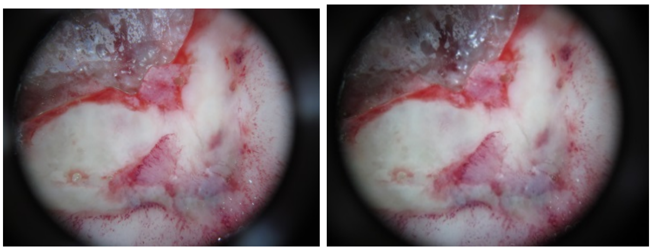

Figure 15. Sample PLANOCELLULAR CARCINOMA, images obtained by diffuse white light and white reflective polarized light

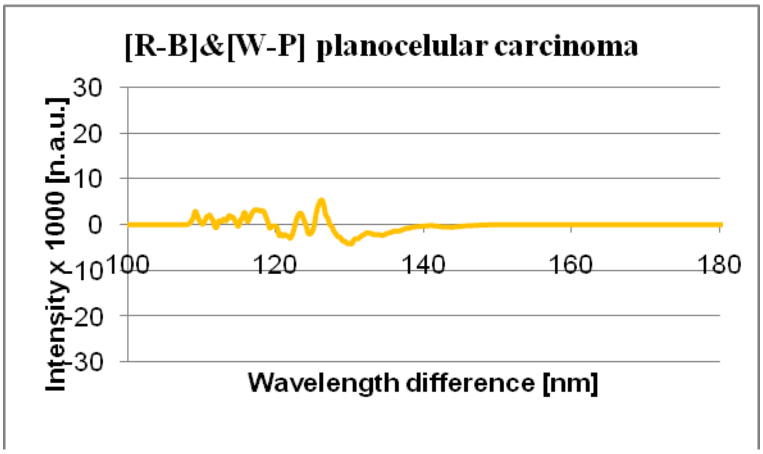

Figure 16. OMIS diagram for Planocellular carcinoma specie of colon tissue

Figures 17, 18 and 19 present OMIS diagrams of non-stained and stained plates of colorectal cancer tissues. These Figures of non-stained plates show a clear peak intensity that is bigger than the value of stained plates, which can be explained with an opinion that the observed tissue has not lost its natural properties. The results obtained with stained and non-stained plates show a significant similarity, which indicates that the staining procedure can be avoided [12]. Benefits include faster and less costly data processing. The most important thing about non-stained plates is that the result does not depend on the procedure of taking smears and staining.

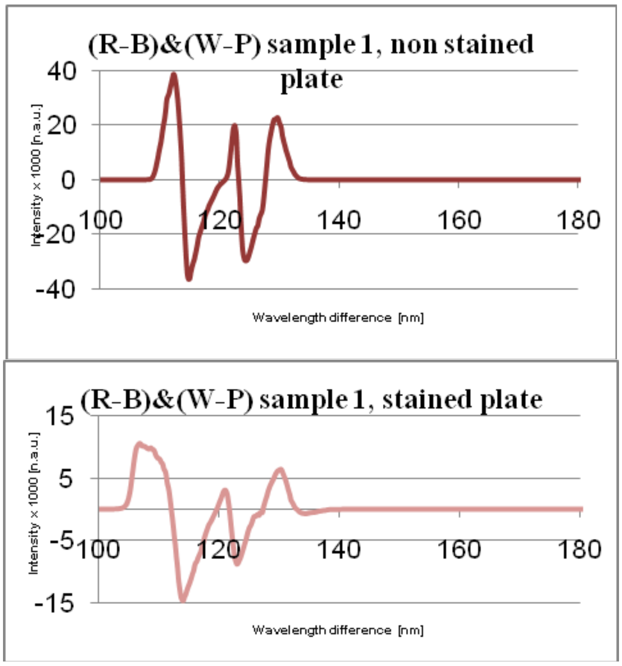

Figure 17. OMIS diagrams of non-stained (up) and stained (down) plates from same tissue sample

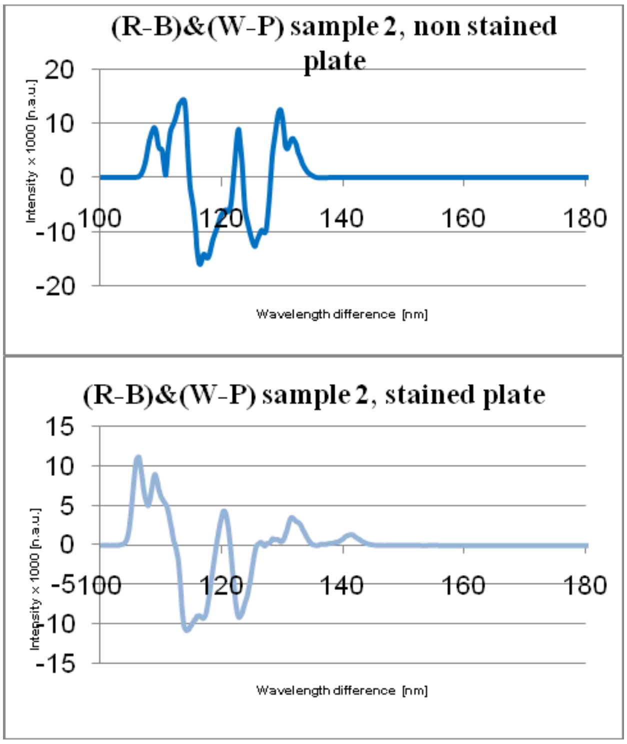

Figure 18. OMIS diagrams of non-stained (up) and stained (down) plates from same tissue sample

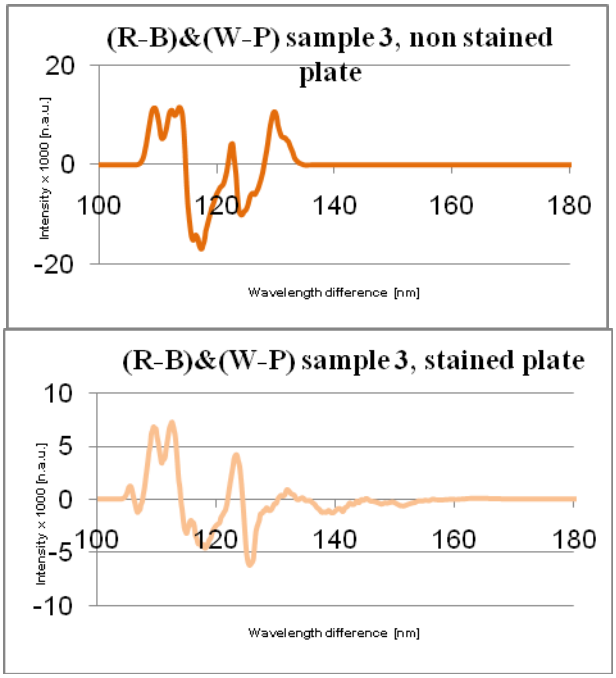

Figure 19. OMIS diagram of non-stained (up) and stained (down) plates of same tissue sample

5. CONCLUSION

The methods that are currently used in the diagnosis of colorectal carcinoma are very expensive and time-consuming. On the other hand, the optical method used for the same purpose, as well as the methods of utility, reduce costs. Some of these methods require specific sample preparation, while others are not portable, and only a few are used *in vivo* [13, 14]. Opto-magnetic imaging spectroscopy, as a noninvasive method, is cheap with portable equipment, it is fast and does not require special sample preparation. OMIS method shows good results in detecting and distinguishing between different types of colorectal cancer *ex vitro*. The next goal is introduction of *in vivo* studies, and the final introduction of the method in health care facilities to improve screening programs and reduce the incidence of new cases and deaths from cancer.

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РАЗЛИЧИТЕ ВРСТЕ КАРАКТЕРИЗАЦИЈЕ КОЛОРЕКТАЛНОГ КАРЦИНОМА УПОТРЕБОМ ОБОЈЕНИХ И НЕОБОЈЕНИХ ПЛОЧИЦА У ОПТОМАГНЕТНОЈ СПЕКТРОСКОПИЈИ

Сажетак: Према подацима Свјетске здравствене организације, карцином је водећи узрок смрти у свијету, а на трећем мјесту је најуобичајенији и најопаснији колоректални карционом са стопом од 17.3/100,000 пацијената. Стога је потребно изнаћи ефективна рјешења за побољшање квалитета и брзине дијагностичких алата који ће моћи одговорити на изазове стратегија превенције који су саставни дио брзе дијагностике код великог броја пацијената. Циљеви истраживања спроведеног у овом испитивању (*in vitro*) су: (1) испитивање могућности примјене оптомагнетне спектроскопије за карактеризацију различитих врста карцинома дебелог цријева, уз помоћ обојених и необојених плочица, (2) валидација методе, (3) одређивање параметара за истраживање у *in vivo* условима, и (4) примјена у клиничким истраживањима како би се побољшала ефикасност и добиле поузданије дијагнозе. У овом раду представљамо оптомагнетну спектроскопију (OMIS) као нову оптичку методу за диференцирање различитих врста карцинома дебелог цријева, као и значајне сличности између обојених и необојених плочица на основу узајамног дјеловања свјетлости и ткива. Резултати су показали да се уз помоћ OMIS-а може наћи разлика између колоректалног и других врста карцинома дебелог цријева, као и доказати да је могуће избјећи (бојење) плочица.

Кључне ријечи: дебело цријево, обојене и необојене плочице, оптомагнетна спектроскопија, снимање.

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