Reflectance polarization \textit{ex vivo} measurements of gastrointestinal carcinoma lesions for cancer diagnostics

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Abstract. The use of polarized light for biomedical diagnostics of skin and mucosa has already a long history. Prior studies have shown a contrast enhancement of the polarimetric values and in images of cancerous and healthy zones tissues. In the present study, reflectance-geometry polarization measurements were made with gastrointestinal tissue (GIT) samples containing normal and neoplastic mucosa. We investigated \textit{ex vivo} the characteristic differences in the polarimetric parameters, including Stokes vector elements, degree of polarization, azimuth and ellipticity angles of cancerous and healthy colorectal tissues. A model PAX1000VIS/M polarimeter (400 – 700 nm, ThorLabs Inc.) was used in reflectance mode to obtain information about these polarimetric parameters of the GIT samples investigated. The most significant differences were observed in the long-wavelength region (>600 nm) in the case of circular polarization of the illuminating and probe axes of the measurement unit. Histological analysis was used as a “gold standard” to compare the optical and pathological diagnosis.

1. Introduction
Cancer is one of the most significant social diseases affecting a significant number of patients worldwide. Among those affected by this disease, mortality is significantly higher than that of other diseases. Early diagnosis of cancer is a key factor in improving the survival and morbidity statistics in the patients affected [1].

One of the most common types of tumors nowadays are those of the gastrointestinal tract (GIT), which occupy 3-4 place in the statistics of tumors found annually in patients around the world. Only breast cancer in women, prostate cancer in men and skin cancer in both sexes have higher statistics than GIT tumors. GIT neoplasms are difficult to detect, very often at an advanced stage of tumor development, leading to a poor prognosis of survival for the patient. For example, early diagnosis of
colorectal cancer resulted in a 92% 5-year survival rate for patients receiving therapy, as opposed to those diagnosed at an advanced stage of development (III-IV), with the 5-year survival rate being estimated at less than 40% [1, 2].

These statistics justify the need for the development and testing of new diagnostic approaches, including such based on optical methods of analysis having high sensitivity in early neoplastic changes in the GIT. It is necessary to accumulate new data on the optical characteristics of tumors, as well as their dynamics related to the changes that occur during the tumor development.

With the optical technologies advancement, more non-invasive and highly sensitive methods for the diagnosis of malignant neoplasms are being sought. One of the possibilities for fast and non-invasive detection and differentiation of cancer and pre-cancer formation is the use of optical polarization methods. A characteristic property of polarized light is to scatter strongly in the tissues, with the resulting multiple acts of scattering leading to its depolarization. Malignancies have been found to depolarize light to a higher degree compared to benign and healthy tissues. This difference allows one to analyze biological tissues by examining changes in the polarization properties of light passing through them [3, 4].

The advantage of methods using polarized light is that obtaining images does not require additional contrast agents, the radiation is non-ionizing and it is possible to work in a non-invasive, non-contact and real-time manner. The prospects that polarized light offers in the field of dermatology and endoscopic diagnostics for obtaining important information about the condition of the biological tissues studied are the reasons that prompted a number of authors to conduct research in the field [5].

However, at this stage, some studies produced different and even conflicting results concerning the alterations observed in tumor vs. normal tissues. For example, Antonelli and co-workers [6] studied colon cancer using a multispectral Mueller polarimetric imaging and light system in the 500 – 700 nm wavelength range. They concluded that tumors in the early cancer stages are less depolarizing than healthy tissues. Also, using the same polarimetric system, they concluded that by analyzing the values of the degree of depolarization, the stage of cancer development can be determined and the effect of the applied radiotherapy on the treated patients can be assessed [7]. On the contrary, Ahmad et al. [8] compared the polarization parameters between healthy and adenocarcinoma tissue of the human colon using Mueller polarimetric system in combination with a polar decomposition algorithm and polarized light with a wavelength in the range 425 – 725 nm. They reported that for all six polarimetric parameters observed, the values increased in adenocarcinoma, compared to the healthy tissue for all used wavelengths.

Pierangello and co-authors [9] analyzed cancer and healthy samples from the human colon using a multispectral Mueller polarimeter operating in the visible spectrum within 500 – 700 nm. Their results demonstrated that using ex vivo multispectral Mueller monitoring, different tissue samples can be distinguished and the stage of tumor development can be determined as well.

Due to the convenience of using polarimetry and the results achieved through it, a number of authors have described methods for the development of polarimetric devices for measurements and their calibration [10, 11].

In our work, we applied polarimetric measurements to the evaluation of normal tissue, benign polyps and malignant colorectal carcinoma lesions.

2. Methods and materials

After surgical removal, the gastrointestinal tissue samples were divided for polarimetric and histological analysis and the first ones were transported under isothermal conditions and in safe-keeping solution from the hospital to the Biophotonics Laboratory (IE-BAS), where their polarization properties were investigated in reflectance geometry. All patients received and signed written informed consent. The research was approved by the Ethics Committee of Tsaritsa Yoanna-ISUL University Hospital, Sofia. Seven colon carcinoma and three colon polyp lesions were investigated during this experiment. Five different points from each lesion and five different points from the surrounding “safety margins” of
normal gastrointestinal mucosa were used for accumulation of polarimetric data and their further averaging and analysis.

Figures 1 (a) and 1 (b) present schematically the two arms of the experimental set-up, as follows: 1a – polarization arm, where the incident laser light is circularly polarized and irradiates the tissue sample, and 1b – detection arm, where the partially depolarized light after its interaction with the tissue sample is detected by a polarimeter working in the visible spectral range 400 – 700 nm (model PAX1000VIS/M, ThorLabs Inc.).

![Figure 1](image)

**Figure 1.** (a) Polarizing part of the set-up (b) Polarization detecting part of the set-up.

The set-up consists of:
1. Diode laser at 635 nm with elliptical polarization.
2. Linear polarizer set to transmit maximum radiation from the laser source.
3. λ/2 plate tuned for vertical polarization.
4. λ/4 plate oriented at 45 degrees relative to the vertical polarization to obtain a circular polarization at the output. Here it is necessary to specify that circular polarization is used because it is depolarized to a greater extent compared to the linear one, which makes it more sensitive for the needs of the comparative analysis between healthy and tumor tissue in the experiment. The use of circular polarization is based on previous studies of the group of IE-BAS [12].
5. Lens focusing the light beam on the sample.
6. Sample.
7. Lens with 10× magnification, capturing the reflected light and directing it to the polarimeter.
8, 9, 10 Schematic diagram of the polarimeter
11. Polarimeter "Thorlabs PAX5710VIS-T", with operating range 400 – 700 nm [1].
12. Computer.

The polarimeter used in the present experiments measures the Stokes parameters S1, S2 and S3 in real time; they are normalized to S0. The following polarimetric values are determined from them: ε – angle of ellipticity, θ – azimuth and DOP - degree of polarization.

3. Results and discussion
During our experiments, the diameter of the laser light beam was 1 mm, which allowed us to obtain an illuminated area sufficiently smaller than lesions or healthy areas on the surface of the ex vivo samples. In this way, the multiple points measured on each sample were independent of each other and several independent parameters could be obtained for a given type of tissue from each sample.

The tumor tissue is characterized by a lower optical anisotropy compared to a healthy tissue, which leads to a greater depolarization in areas without developing cancer. The reason for such difference is that when the tumor cells infiltrate into healthy areas, the anisotropy of the extracellular matrix is strongly reduced due to its disruption. Therefore, we expected a difference in the measurements in the polarimetric parameters of healthy tissues compared to those of tumor tissues. Benign GIT lesions, such as polyps, should have intermediate polarimetric parameters, but closer to the ones of healthy tissues, as the extracellular matrix in their case is not significantly disturbed by the lesion growth process. As the
level of anisotropy is expected to be related mainly to the alterations in the morphology of the extracellular matrix, we could expect that benign lesions are suboptimal to be differentiated from healthy tissues using polarimetric parameters.

Figures 2 (a), 2 (b) and 2 (c) present averaged values of the major polarimetric parameters for, respectively, normal, benign and malignant lower gastrointestinal tract tissues.

![Figure 2.](image)

**Figure 2.** (a) Stokes parameters with their uncertainties (b) Degree of polarization, DOP, with its uncertainty (c) Azimuth, \( \theta \), and angle of ellipticity, \( \epsilon \), with their uncertainties and histological states of the tissue samples investigated. In red are the data for tumor (T) GIT carcinoma; in green, for healthy (H) GIT mucosa; and in orange, for benign GIT polyp (P).

In figure 2 (a) one can see a significant difference between the Stokes parameters of a healthy tissue and those of a tumor tissue. Higher values are observed for \( S_1 \) and \( S_3 \) in the tumor tissues, while for \( S_2 \), on the contrary, higher values are observed in the healthy tissue. Their differences are statistically significant (\( p < 0.05 \)). The following graph, 2b, shows that the value of the polarization degree of the tumor tissue is higher than that of the healthy tissue. The azimuth and angle of ellipticity reveal a clear distinction between the healthy and tumor areas. For the azimuth, the value of the parameter is higher in the healthy tissue, while for the angle of ellipticity the situation is the opposite.

We can, therefore, conclude that the method is reliable for distinguishing healthy vs. tumor tissue by comparing Stokes parameters, degree of polarization, azimuth and angle of ellipticity.

The same approach was applied to comparing the parameters of tumor tissues and polyps. The graphs 2a – 2e show that there is no overlap of the uncertainty boundaries, with the exception of the degree of polarization. This is again an indication of the applicability of this method to distinguishing benign polyp vs. malignant tumor tissue. We thus conclude that it can be used for early diagnosis of tumor formation in the early stages of its development, as well as for differentiating between benign and malignant tumors, which is an important task in the clinical practice when a therapeutic plan is developed.

However, when considering the values of the parameters obtained from polyp and healthy tissue, it is seen that most of them overlap within their uncertainties. Exceptions are the Stokes \( S_3 \) parameter and the angle of ellipticity; as expected, we conclude that the method is not sensitive enough to distinguish benign tissue from a healthy one. The difference between the morphological compositions of these two conditions is much smaller than that between tumor and healthy tissue, which leads to less pronounced differences in their polarimetric characteristics.

4. Conclusions
The initial studies reported showed a high sensitivity of the method for differentiation of healthy vs. tumor tissue and tumor vs. polyp. Based on them, we believe that this specific optical scheme for tissue polarimetric analysis aimed to differentiate between cancerous and healthy tissues is promising for practical applications and will be of assistance in accumulating statistics to assess the specificity of the method.
The experiments presented were performed in a reflection mode. The measurement is in real time, and the samples are a few centimeters thick, which mimics conditions close to those of in vivo diagnostic procedures. Using reflection geometry for light detection from the sample, the depolarization of the radiation observed is significant, which provides data suitable for studying and comparing different tissue states.

The differences found in the values of the polarization parameters for healthy and tumor formations indicate changes during tumor growth. The ability to distinguish small changes in the polarimetric values of areas with different histological conditions could allow one to diagnose a malignancy at an earlier stage of its development, which is a key factor in increasing patients’ survival. On the other hand, polarimetric analysis of tissue could be of benefit to physicians in the event of difficulties in definitively diagnosing the presence of a malignancy.

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