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Event: Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments 2016, 2016, Wilga, Poland

Polarimetric characterisation of histological section of skin with pathological changes

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ABSTRACT

This work focuses on the further development of the optical methods based on polarization detection to improve the reliability of the results of evaluation of structural changes in biological tissues. The paper presents an experimental study of tissues with pathological alterations to assess the capacity of differentiation of pathological conditions when using averaged local Mueller matrix elements. Experiments were carried out in direct scattering mode in histological samples of human epidermis. The practical significance of the results is the widespread application of the methodology for laser polarimetric analysis of structural changes in anisotropic tissues to identify and assess the degree of pathology in medical diagnosis of skin diseases.

Keywords: laser polarimetry, biological tissue, Mueller matrix, depolarization, optical anisotropic properties

1. INTRODUCTION

The development and improvement of methods and tools for early detection of structural and pathological changes in biological tissues is of fundamental importance in modern medicine. On the cellular level, a significant number of biotissues have ordered structures, which generate optical anisotropic properties (optical activity, birefringence, dichroism, and others). Therefore, determination of polarization characteristics provides an opportunity to investigate structural changes due to various pathologies. Therefore, a promising area of the optic instrumental methods in the diagnosis of tumors in biological tissues is to develop detection means based on the use of polarized light in both visible and near-infrared spectral ranges ¹⁻⁴.

The significant progress of polarimetric evaluation include new areas such as polarization-sensitive OCT, optical polarimetry. Despite notable success in biological polarimetry, the introduction of laser polarimetry for biotissue evaluation is still in experimental stage. Besides, the differences in the methods of preparation and experimentation lead to difficulty of standardization and results interpretation 5 .

2. MATERIALS AND METHODS

Experimental research of the polarimetric method for the differentiation of pathological changes in anisotropic structures of biological tissue was performed using the adaptive computer system of laser polarimetry of Faculty of Radio Physics, Electronics and Computer Systems of the Taras Shevchenko University⁶.

The light source is a He-Ne laser with wavelength λ =632.8 nm. The polarized beam from the source passes through a collimator that generates a parallel expanded beam of diameter 4÷10mm. For eliminating the scattered and diffracted

Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments 2016, edited by Ryszard S. Romaniuk, Proc. of SPIE Vol. 10031, 100313E · © 2016 SPIE CCC code: 0277-786X/16/\$18 · doi: 10.1117/12.2249373 light, a spatial filter (orifice diameter of 50 μ m.) is used ⁷. The formation of the polarization states of channel I is achieved by a modulator, which is composed of a polarizer and a phase plate. The converted elliptical polarized beam is scattered by the tissue sample, which is mounted on a holder with a two-coordinate micro-positioner. The scattered radiation enters the detection channel II, which consists of a rotating phase plate FP, the analyzer A and the photodiode PD. The rotational components of the polarization components of the polarimeter are equipped with positional encoders (optocouplers). The converted output signal is recorded and processed in a personal computer with specialized software.

The procedure for determining the parameters of polarization of the tissue sample is based on the linear interaction of light with the thin sample of tissue within the Mueller matrix formalism ⁸. The polarization of the probing beam, emerging from channel 1 and the detected beam in channel 2 is characterized by the Stokes vector S.

$$S = \begin{bmatrix} S_0 & S_1 & S_2 & S_3 \end{bmatrix}^T = \begin{bmatrix} I_h + I_v & I_h - I_v & I_+ + I_- & I_R - I_L \end{bmatrix}^T ,$$
(1)

where I_h , I_v , I_+ , I_- is the intensity of light, transmitted through a linear polarizer oriented at angle 0^0 , 90^0 , 45^0 and -45^0 respectively; and I_R , I_L is the intensity of light transmitted through a right circular and left circular polarizer ⁹.

The effect of a biotissue sample on the Stokes vector of probing light can be described using a 4x4 transformation matrix, called Mueller matrix and the effect of multiple optical elements on light polarization is described by the matrix product $S^{out} = M_3 M_2 M_1 S^{in}$ while the Mueller matrix M_i of the elements, successively traversed by the light beam, are arranged in reverse order.

Therefore, at normal incidence of the probing, light the polarization characteristics of the sample are represented by the matrix expression ^{10.}

$$S^{\text{out}} = M_A M_{FP} M_{BT} M_{FP} M_P S^{\text{in}}$$
(2)

where M_A , M_{FP} , and M_P are the polarizer components Mueller matrix (analyzer, phase plate, polarizer); M_{BT} is the Mueller matrix of biotissue sample local area.

The analyzer output intensity varies according to a periodic law, thus, the change in intensity can be expressed in terms of the sum of the corresponding spectral components of the Fourier transform ¹¹:

$$I(t) = a_0 + \sum_{k=1}^{n} (a_k \cos(k\omega t) + b_k \sin(k\omega t))$$
(3)

where a_k , b_k are the harmonic amplitudes and ω fixed phase plate rotating frequency.

The complete Mueller matrix M_{BT}^{Σ} of an optically thin BT cut is generated based on the superposition of the local coordinate cell matrices $M_{BT}^{m,n}$, taking into account their spatial distribution (m, n) in the plane of the sample ¹².

Figure 1. Tissue sample (1), laser source (2), local measured points (3)



Experimental studies were carried out in vitro in epidermis thin histological cuts ($20 \div 50$ mm) with papilloma and melanoma (at 2nd stage by Clark). Epidermis cuts (24 pcs.) were fixed on glass slides 1.5 mm thickness in Canadian balsam. Linear tumor sizes ranged from 8 mm to 20 mm. Samples were taken from the forearm European men aged 55-

65 years and were analyzed one day after preparation.

3. RESULTS AND DISCUSSION

Table 1 shows Mueller matrix elements by statistically-averaging local area measurements of thin cuts (40 microns) of epidermis melanoma and papilloma and Fig. 4 indicates the absolute value of their differences. Table 1 shows that for both types of cut (with papilloma and melanoma), the elements of the main diagonal of the Mueller matrix are close to 1. This fact shows that in both cases, the samples significantly do not depolarize the incident light. For this reason, depolarization cannot be a characteristic which allows to distinguish tissue pathological conditions. For thicker cuts of epidermis depolarization could have a more pronounced difference for samples with melanoma and papilloma that will serve as additional information parameter. However, the used method of fixation does not allow quality plane-parallel cuts thicker than 100 microns.

Figure 4 shows that the direct analysis of the elements of the Mueller matrix is possible through the elements 24 and 42. These elements reveal a capacity for differentiation of pathological conditions.

Mueller	Melanoma	Papilloma	Mueller	Melanoma	Papilloma
matrix			matrix		
element			element		
m11	0.368	0.367	m31	0.012	-0.006
m12	0.008	0.005	m32	0.002	-0.012
m13	0.001	-0.010	m33	0.988	0.967
m14	-0.001	-0.001	m34	0.000	0.022
m21	-0.005	-0.003	m41	0.005	0.000
m22	0.991	0.979	m42	0.011	-0.086
m23	0.005	0.016	m43	0.002	-0.015
m24	-0.013	0.078	m44	0.987	0.956

Table 1 Statistically-averaged Mueller matrix elements of epidermis thin cuts (40 microns)



Figure 2. Difference in absolute value of Mueller matrix elements for Melanoma and Papilloma cut samples.

4. CONCLUSIONS

The analysis of the Mueller matrix in the direct scattering mode shows that some elements have enough to analyze morphological changes in biological objects sensitivity. Depolarization of the light probe is relevant for studies of samples greater than 100 microns, however, this requires a better method of preparation of the samples. A more detailed study would require the expansion of the samples polarization properties in different types of anisotropy.

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