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BIOMEDICAL APPLICATIONS OF ULTRASHORT PULSED LASER POLARIMETRY

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Abstract. This paper is focused on the experimental analysis of different pathologically modified tissues, using a polarization sensitive imaging technique with a pulsed femtoseconds laser. The experimental set-up used permits the acquisition and recording of 24 polarization images, with the possibility of extracting an important amount of information regarding the tissue inhomogenous structure and any pathologically induced behaviors of the sample. The colour histograms corresponding to these images can also be obtained.

Key words: light polarization, pulsed femtoseconds laser, polarization sensitive imaginery.

1. INTRODUCTION

The combination of the polarization imaging techniques with the classical optical coherence tomography (OCT), represent a new and fast growing method of optical investigation [1–5].

Due to the fact that most of the real biological tissues have an optically inhomogeneous structure, any changes produced in the optical polarization state of a light beam passing through such samples (or reflected by them) can reveal important information about the respective biological tissue.

Once the polarization sensitive images are obtained, the best way to have access to the optical polarization properties of the samples involved in the investigations is to compute their Mueller matrices, therefore the result is a Mueller optical coherent tomography (MOCT).

Since the birefringence is related by different biological compounds (collagen, muscle fibers, keratin, etc.), using a pulsed femtosecond laser as radiation source, the optical contrast for all the polarization sensitive images recorded is significantly improved.

2. EXPERIMENTAL SET-UP

The optical scheme of the polarization sensitive imaging system [6] used in the acquisition of the polarization images, is presented in Fig. [1].
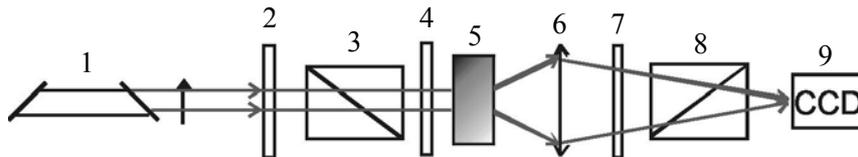

Fig. 1 – Optical scheme used for the investigation of the polarization sensitive images of the sample. The numbers represent: 1 – laser; 2, 4 and 7 – quarter-wave plates; 3 – polarizer; 5 – sample; 6 – microscope objective; 8 – analyzer; 9 – CCD camera.

The optical scheme uses as radiation source a Tsunami femtosecond laser from Spectra-Physics, working in pulsed regime, with the pulse length shorter than 100 fs and the medium power adjusted around 50 mW, both values measured before entering in the optical set-up. The laser operates in IR at a wavelength of 800 nm.

The frequency of the pulse repetition was 80 MHz and the optical polarization state for the laser beam at the entrance in the optical set-up was linearly vertical polarized.

The polarization illuminator consists in two quarter-wave plates and a polarizer, which provides a laser beam with the polarization parameters: α – the azimuth between $[0^0, 180^0]$ and β – the ellipticity between $[0^0, 90^0]$.

As a result, the reduced Stokes vector for the incident laser beam on the sample (biological tissue) will be:

$$\hat{S}_0 = \begin{pmatrix} 1 \\ \cos 2\alpha \cos 2\beta \\ \sin 2\alpha \cos 2\beta \\ \sin 2\beta \end{pmatrix}. \tag{1}$$

The polarization sensitive images are projected by the means of a microscope objective onto the plane of a light-sensitive CCD camera, with the minimum number of pixels 800×600, after passing the polarization state analyzer system composed from a quarter-wave plate and an analyzer.

A picture of the holographic table where all the optical components are installed is presented below:
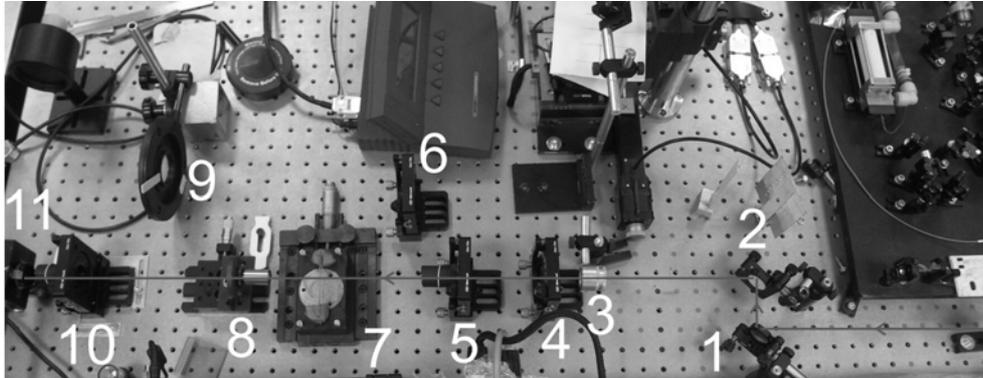
Fig. 2 – Photography of the optical polarization components on the holographic table.

In this photography the numbers represent: 1 and 2 are two mirrors used to direct the laser beam onto the optical axis of the set-up, 3 is an attenuator (10×) used to protect the CCD camera from the high intensity pulsed laser beam. 4, 6 and 9 are quarter-wave plates, 5 and 10 are the polarizer, respectively, the analyzer, 7 is the sample slide mounted on a stage with adjusting micrometric screw, 8 is a microscope 10× objective and 11 is a CCD camera.

The preparation of the biological tissues investigated in this paper, was made by the method of cutting by cryotom from the same frozen bulk tumor sample (renal tumor). The medium width of sample slices was 5 μm.

Slides were selected to have both tumor tissue and normal tissue. Using the micrometric screw, the slides were translated transversal to the system optical axis, so that only certain areas of the samples to be investigated.

The polarization sensitive images of optically thin histological sections of renal tumor, can be obtained and recorded with the CCD camera for different configurations, by rotating both the quarter-wave plates 6 and 9 (from the photography in the Fig. [2]) as well as the polarizer 5 and analyzer 10.

3. EXPERIMENTAL RESULTS

In the Fig. 3 are presented the polarization sensitive images recorded using the set-up described before.

As it can be seen, from these 24 polarization images, 4 are obtained for crossed polarizer and analyzer and therefore are dark images (0 – 90, 90 – 0, 45 – 135, 135 – 45).
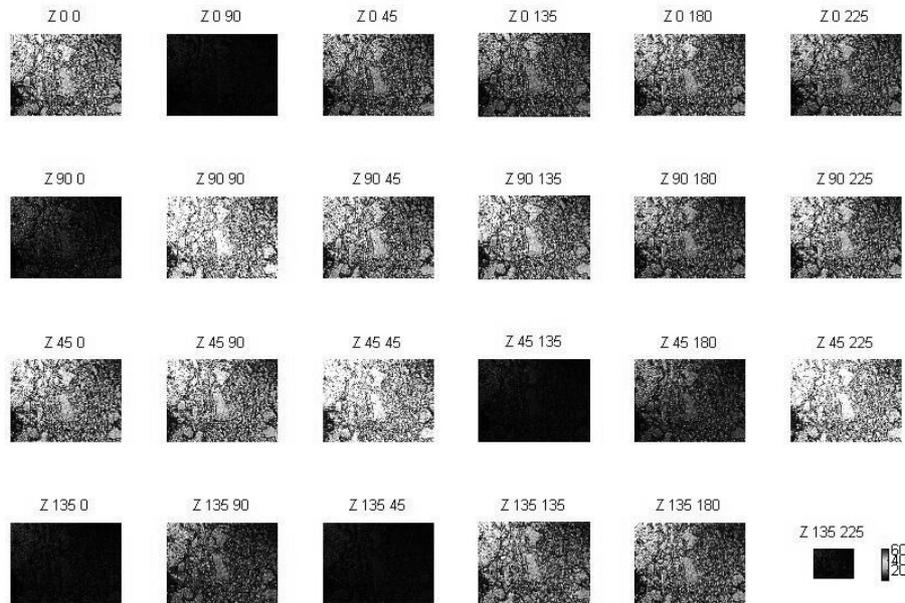
Fig. 3 – The polarization sensitive images obtained with a pulsed femtosecond laser beam.

The light intensity in every point of such a polarization sensitive image can be computed using the following expression:

$$I = I_0 \left[\cos^2 \left(\alpha \pm \theta \right) + \text{tg}^2 \beta \sin^2 \left(\alpha \pm \theta \right) \right] . \tag{2}$$

In the relation above, θ – represent the orientation angle between the passing axes of the polarizer – analyzer system.

From any polarization sensitive image obtained, the graphical 2D-representation of the Mueller matrix corresponding to the particular configuration chosen can be obtained, simply by computing all the corresponding pixels of that polarization image [7].

The product between the Stokes matrix of the polarization illuminator, provided by the relation (1) and the computed Mueller matrix of the sample must be equal to the Stokes matrix of the image collecting system.

The sample area chosen for investigation, has a particular morphology, as it can be seen from the Fig. 4 below.

The image above possesses a very good contrast (much higher than using a He-Ne laser, for example, as a radiation source). The area in the center presents a strong anisotropy and it is surrounded by some isotropic areas.

On the other hand, the image visually proves that, in this case of a renal tumor, we have a bigger extent of anisotropy than in the case of the healthy renal tissues.

Fig. 4 – The polarization sensitive image of a renal tumor sample illuminated
with a femtosecond pulsed laser beam.

In Fig. 5 we present the histograms computed using an usual image
processing software, corresponding for the following configuration: 0 – 0, 0 – 90, 0
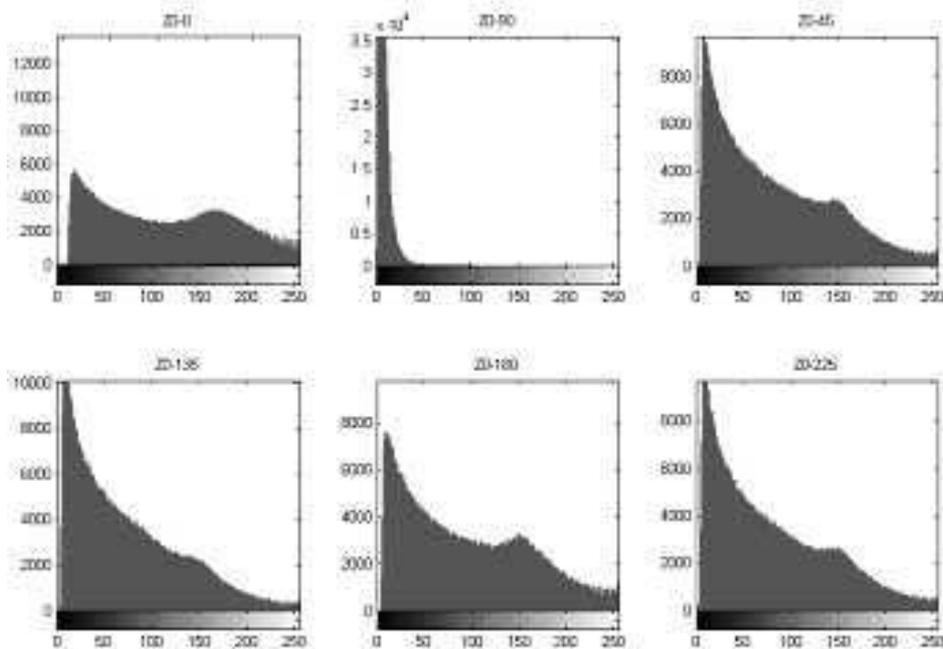– 45, 0 – 135, 0 – 180 and finally 0 – 225.

Fig. 5 – The histograms corresponding to 6 polarization sensitive images.

In comparison with the usual histograms obtained for healthy tissues, which have a narrow intensity distribution, in our case the histograms corresponding to the renal tumor investigated shows a rather more uniform intensity distribution. This fact is due to the local annihilation of the birefringence in the presence of the cancerous tissues.

4. CONCLUSIONS

We have obtained 24 polarization sensitive images corresponding to samples prepared from renal tumor tissues, using as a light source a femtosecond pulsed laser, operating in infrared. The histograms corresponding to some of these images were also calculated.

The good quality contrast of these polarization images combined with the possibility of making a complete mapping of the Mueller matrices, indicates this technique as a new powerful tool in the early detection of any pathological changes of biological tissues.

REFERENCES

1. S. Jiao, L.V. Wang, *Two-dimensional depth-resolved Mueller matrix of biological tissue measured with double-beam polarization-sensitive optical coherence tomography*, Opt. Lett., **27**, *2*, 101–104 (2002).
2. I. Ionita, P. Reisen, *Imaging of dental implant osseointegration using optical coherent tomography*, Proceedings SPIE, BIOS, San Jose, USA, 2009.
3. J.F. de Boer, T.E. Milner, M.J.C. van Gemert, J.S. Nelson, *Two-dimensional birefringence imaging in biological tissue by polarization-sensitive optical coherence tomography*, Opt. Lett. **22**, 934–936 (1997).
4. A.G. Ushenko, O.V. Angelsky, D.N. Burkovets, V.P. Pishak, Y.A. Ushenko, O.V. Pishak *Polarization-correlation investigations of biotissue multifractal structures and their pathological changes diagnostics*, Laser Physics, **10**, 1136–1142 (2000).
5. A.G. Ushenko, D.N. Burkovets, Y.A. Ushenko, *Polarization-Phase Mapping and Reconstruction of Biological Tissue Architectonics during Diagnosis of Pathological Lesions*, Optics and Spectroscopy, **93**, 449–456 (2002).
6. A. Ushenko, S. Yermolenko, A. Prydij, S. Guminetsky, I. Gruia, O. Toma, K. Vladychenko, *Statistical and fractal approaches in laser polarimetry diagnostics of the cancer prostate tissues*, Proc. of SPIE, Volume 2008, Bellingham WA, 2008.
7. O. Toma, E. Dinescu, *Application of the matrix formalism in a Mueller matrix imaging polarimeter*, Rom. Rep. Phys., **60**, *4*, 1065–1070 (2008).