CORRELATION OF OPTICAL METHODS AND BIOCHEMICAL MEASUREMENTS FOR INVESTIGATION MEMBRANE CHANGES OF NORMAL AND MALIGN TISSUES

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Scopul acestei lucrări constă în a reuni oportunitățile tomografiei optice de coerență Mueller pentru bio țesuturi denumita MOCT (Tomografie Coerentă Optică Mueller) cu tehnicile de polarizare, corelare şi fractali ai imaginilor matricei Mueller (MMI) pentru diagnosticarea precoce a tumorilor şi patologiilor degenerative a bio țesuturilor. Acest lucru constituie o analiză complexă a structurilor optice şi geometrice şi a arhitecturilor referitoare la diferite nivele de ierarhie fractală şi multifractală. Pentru a testa validitatea acestei metode s-au efectuat şi măsurători biochimice (stresul oxidativ) a unor tumori maligne induse experimental.

The aim of this paper is to join together the opportunities of Mueller optical-coherent tomography for bio-tissues, named MOCT (Mueller optical coherent tomography), with the techniques for polarization, correlation and fractals of MMI (Mueller matrix images) for early diagnosis of cancerous and degenerative pathology of BT (bio tissues). This was realized as a complex analysis of optical and geometrical structures, and of their architecture referring to different levels of hierarchy- fractals and multi-fractals. To attest the validity of the optical method, biochemical measurements ((oxidative stress) of experimentally induced tumors were performed.

1. Introduction

The novelty of this optical method is provided by considering two procedures of bio tissues diagnosis: fractal analysis of object-image planes and laser polarimetry of bio tissues matrix [1], [2], [3]. This synthesis might provide a new method for pre-clinic diagnosis. The following steps have been considered:1) obtaining all MMI of BT, 2) finding punctual statistic parameters of total OT (distribution after coordinates of equally oriented fractal planes) and PT(distribution after coordinates of birefringence index for bio-fractals

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substances), 3) finding correlation functions of total OT, PT and MMI, and 4)fractal analysis of OT, PT and MMI.

Bio-tissues are usually available as objects with crystalline-amorphous matrix. The amorphous component is optically anisotropic[1]. Crystalline components are found as architectonic nets (AN) and are optically active or bi-refringent. AN structure may be considered as fractal-levels or multi-fractal levels of the matrix [4],[5]. Fractal level is represented by the AN parts, with a space-ordered structure of microfibres [6]. Such features are encountered in normal tissues, as for example: elastyne induces collagen-additives in conjunctive and muscular, tissues of reproduction organs and osteons in bone tissues.

The aim of this paper is to use techniques for early pre-clinical diagnosis and reveal pathological and degenerative changes in bio- tissues architectonic net, by using the opportunities of MOCT of forecasting the fractal, correlation and statistic parameters of OT, PT and MMI. An interconnection between morphological structure of membrane tissue and polarization image was found.

The results obtained when using this method are compared to those given by biochemical methods. As known, in cancer-induced tumors a dramatic increase of free radical oxygen production is noticed and this one may be determined by oxidative stress reactions.

2. Biochemical measurements and results; experimental procedure

In order to determine fatty acids effects on cancer-induced tumors we used the following experimental procedures.

1. A lot of healthy Wistar male rats were fed for 10-12 days with fatty acids (linoleic, arahidonic and a mixture of them).
2. Cancerous tumors were inoculated into Wistar rats by under-skin grafts.
3. After 7,10,14, and 21 days from tumor inoculation the rats were sacrificed and samples from different tumor tissues were taken in order to be subjected to different investigations.
4. Typical biochemical parameters such as lipid peroxides, caeruloplasmins, thiols and oxidative stress indices were determined in each case.

An experimental hepatic tumor RS1 has been chemically induced by administrating 2-acetylaminofluoren into rats' food. Wistar mature rats of 200-250 grams, clinically healthy have been used. The tumor has been experimentally induced by under skin grafts. After 7, 10, 14, 17 and 21 days from inoculation an animal has been sacrificed and samples of hepatic and pancreatic tumor tissues have been prevailed.

The oxidative stress index (OSI) was estimated according to the following relationship

$$OSI = \frac{P_x C_p}{SH},$$

where P_x are the lipid peroxides (μmoles MDA/100mL ser), C_p the caeruloplasmin estimated in (UI) and SH the albumine thiols (μmoles / L).

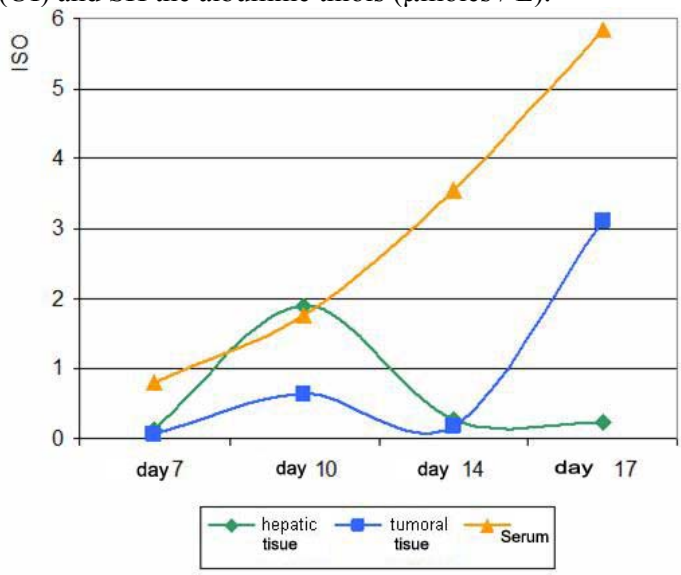

Fig. 1. Oxidative stress index (ISO) changes in serum, hepatic and tumoral tissues

In Fig.1 and Table I we give the time evolution of the oxidative stress when the lot of rats was fed with a mixture of linoleic and arachidonic acids

Table I. Values of oxidative stress index in serum, hepatic and tumoral tissues (lot fed with linoleic and arachidonic acids)

Biological sample	Day 7	Day 10	Day 14	Day 17
Hepatic tissue	0,14	1,90	0,28	0,23
Tumor tissue	0,07	0,64	0,19	3,11
Serum	1,34	1,77	3,54	5,85

The dramatic increase of the oxidative stress index for this lot of rats is due to a severe oxidative stress induced by the tumor. In hepatic tissue and serum the oxidative stress index registered smaller values in the presence of experimentally induced tumor. This result shows that when animals (in this case, rats)exhibiting cancerous tumors are fed with fatty acids important changes of the membrane structure and biological parameters have to be expected [7]. According

to Table I after 17 days from tumor inoculation the oxidative stress index presents an important increase in the serum and tumor tissue.

3. Optical measurements

3.1 Theoretical basis of the optical model for the bio-tissues

The optical model for a bio-tissue is based on the fact that bio-tissues might be represented by bi-component amorphous-crystalline structures.[3], [7]. The BT geometry displays hierarchic structures (microfibrils, fibrils, fibres, fascicules). These structural elements are discrete and display a repeatability at a scale comprised between $1 \div 10^3$ μm.

The structural elements are optically similar to an uni-axial crystal. The directions of the optical axes are determined by the angles ρ of the fibrils from BT layers, while the birefringence values Δn are determined by the substance anisotropy [8].

The *Mueller* matrix of the BT layer which determines the polarization properties is:

$$\{M\} = \begin{Vmatrix} 1 & 0 & 0 & 0 \\ 0 & m_{22} & m_{23} & m_{24} \\ 0 & m_{32} & m_{33} & m_{34} \\ 0 & m_{42} & m_{43} & m_{44} \end{Vmatrix}$$

where: $m_{22} = \cos^2 2\rho + \sin^2 2\rho \cdot \cos\delta$

$$m_{23} = m_{32} = \cos 2\rho \cdot \sin 2\rho \cdot (1 - \cos\delta)$$

$$m_{24} = -m_{42} = -\sin 2\rho \cdot \sin\delta$$

$$m_{33} = \sin^2 2\rho + \cos^2 2\rho \cdot \cos\delta$$

$$m_{34} = -m_{43} = \cos 2\rho \cdot \sin\delta$$

$$m_{44} = \cos\delta$$

and $\delta = (2\pi / \lambda)\Delta nL$ is phase difference due to the optically anisotropic fibril; L the fibril thickness and λ is the laser wavelength [9].

3.2 Experimental procedure, data processing and results

A number of 6 samples consisting of malign tissue were prevailed from the tumor and hepatic tissues in different stages of evolution: 7, 10, 14, 17, 21 and 30 days after tumor inoculation. They were cut with a 5 μm cryomicrotom and then fixed on the microscope lamellas. Bidimensional images of bio-technical

have been obtained with a CCD camera, in different configurations (polarizer and analyzer positions)

The optical measurements revealed that the cancerous tissues were sensitive with respect to the rotation of polarization plane. In advanced levels of cancers a dramatic deterioration of these structures take place; these cannot be revealed by the laser polarimetry method.

In Fig.2 we give, for exemplification, the images obtained for the six above-mentioned samples prevailed in different stages of evolution. As it may be seen, the samples display different degrees of birefringence. In the last stage of evolution of the cancerous tumor, the tissue is dramatically destroyed being practically amorphous.

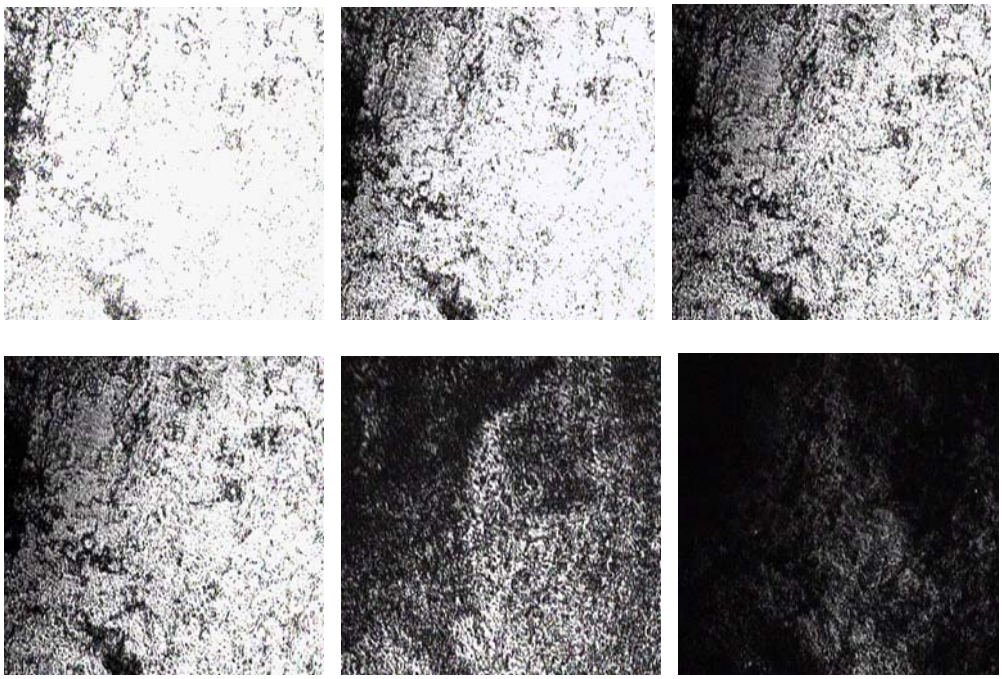

Fig. 2 Sample images prevailed after 0, 7, 10, 14, 17, and 21 days after tumor inoculation (obtained for crossed polarizers).

Using the same experimental equipment described in [10,11] we obtained for each sample 24 images corresponding to Mueller matrix for different configurations of polarimetric system.

The 24 images (corresponding to Mueller matrix) of each above-mentioned samples have been processed using three programs developed under MATLAB: 1) First we digitalized each image in 0-1 forma, according to Matlab

programme, 2) We visualized the images in Matlab format, 3) For each image we calculated the corresponding histograms. These histograms give distribution of the amorphous and crystalline components.

The ideal histogram is harmoniously layed from the left part (shadow area) until the right part (light area), without maxima towards extremities and without being crowded excessively at any borders. The ideal histogram does not present any „wholes" especially towards the borders. Underexposed images display low light and main black tones and the histogram seems to be shifted to the left. In a severe underexposed histogram white and light grey areas are missing. The shadow details are lost and cannot be found again.

In Fig. 3 three images of prevailed samples and the corresponding histograms are shown. They correspond to samples collected in the first day and after 10 and 21 days from tumor inoculation.

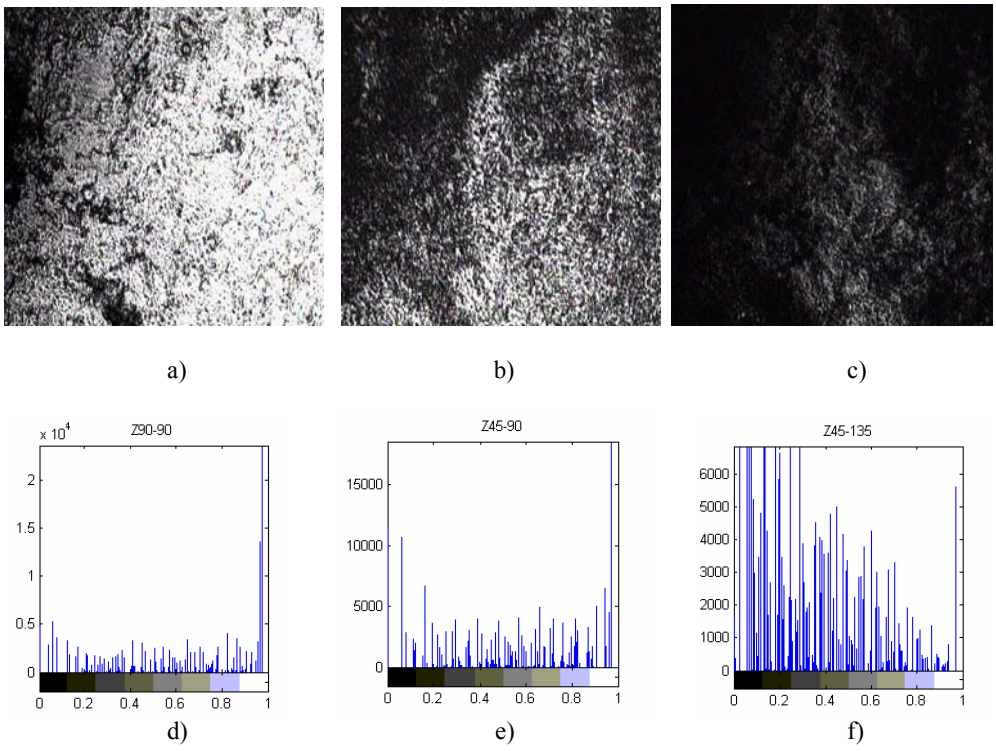

Fig.3 Microstructures of samples collected in the first day (a) and after 10 (b) and 21 (c) days from tumor inoculation and the corresponding histograms (d, e, f)

The image shown in Fig.3a corresponds to a birefringent sample in which the amorphous and crystalline components are rather uniform spread; it corresponds to a normal tissue. Its histogram is given in Fig.3d.

The image given in Fig.3b is less birefringent indicating an increase of the amorphous component. It belongs to a sample prevailed after 10 days from tumor inoculation and shows the presence of a dangerous (cancerous) tissue. Its histograme is given in Fig 3e.

The last image given in Fig.3c is rather nonbirefringent, indicating that the amorphous component is a dominant one. This shows that after 21 days after tumor inoculation the amorphous component is spread chaotically in the cancerous tissue. Its histogram is given in Fig.3f. In this case no information may be acchived when using polarized light.

4. Conclusion

Our results reveal the possibility of using the Mueller matrix method for obtaining information on polarization properties of bio-tissues. It relays on the fact that the bio-tissues have structures with two amorphous-crystalline components and display optical anisotropy .

When a malign cell is included in this structure the anisotropy is changed or even destroyed. This change may be visualized by optical methods and proved to be correlated with data referring to oxidative stress parameter.

The fractalometry techniques based on MOCT proved to be an useful tool in pre-clinic diagnosis of pathological changes. We include here the malign changes, since the cancerous cells are loosing their anisotropy and develop chaotically.

The experimental results underlined that after 21 days from tumor inoculation the anisotropy is lost and no experimental optical data may be obtained.

Our results indicate that fatty acids administration in tumor development dynamics may influence the membrane structure and biochemical parameters. After 17 days from tumor inoculation the oxidative stress index presents an important increase in the serum and in tumoral tissue.

R E F E R E N C E S

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