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Methods and means of polarization parameter control in biotissue imaging polarimetry

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ABSTRACT

The given paper considers experimental methods and means of laser polarimetry that can be used for control of polarization parameters of biotissues, in particular, in case of determination of the degree of pathological changes in human skin. The scheme and operation of universal automated imaging polarimeter are considered. The results of experimental research of Mueller matrices and corresponding polarization characteristics for thin cuts of human skin with visualization by means of vector analysis are presented.

Keywords: Mueller matrices, imaging polarimetry, anisotropy, biotissue

1.INTRODUCTION

Recently more and more attention is paid to methods and means of optics of scattering media, based on analysis of interaction of laser polarized radiation with the object [1-5]. Investigation of polarization characteristics of the field of scattered optical radiation is the basis of laser polarimetry methods, which are widely used nowadays [6-8]. Characteristic feature of laser polarimetric systems is the ability to take into account vector structure of certain medicobiological media [8]. All this stimulated the development and improvement of methods of matrix optics of light scattering objects and media, these methods, successfully combine the possibility of simultaneous obtaining of information regarding spectrophotometric and polarization characteristics of radiation scattered by the object.

Analysis of polarization state in case of small angle multiple scattering is rather perspective for optical diagnosis of certain biological media and tissues (surface layers of the skin, multilayers of cells, etc.) and can serve as alternative to temporal, phase-frequency methods of research, circuits of optical medical tomography, etc. [2-4]. The existing methods of direct measurement of intensities of scattered of polarized components allowed to generalize and classify a number of phase-inhomogeneous objects [2].

While studying separate biotissues, information parameters, which characterize their structure, can be the degree of depolarization of initially polarized light, as well as the character of polarization transformation from one kind into another [3,5] . However the complexity of mathematical apparatus intended for analysis of interaction of polarized radiation especially with phase-inhomogeneous multiply scattered media makes experimental research of polarimetric parameters of various types of biotissues (structural and non-structural, amorphous, optical-active, etc.) by means of automatic imaging polarimetry actual. Such investigations some case are the most efficient way of obtained measuring information regarding structural changes in biotissues [3,4,8].

2. METHODS

Known methods of polarimetry, being applied for investigation of polarization characteristics of various anisotropic media (crystal optic, transparent tissues of eye, biological liquids etc.) are based on measurement of parameters Stocks vector optical radiation **S**. These parameters can be determined experimentally on the basis of six values of radiation fluxes, obtained by means of measurement of light intensity after passing across conversion filters (elements with preset states of polarization) which are located directly in front of photodetector [2,7]. The first of the filters is isotropic, other pass radiation with corresponding to each filter direction of vector **E** oscillations. Thus

$$\boldsymbol{S}=\begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix}=\begin{bmatrix} I \\ I_H - I_V \\ I_{+45^\circ} - I_{-45^\circ} \\ I_R - I_L \end{bmatrix}, \tag{1}$$

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where I – intensity of input radiation; I_H, I_V, $I_{+45°}$, $I_{-45°}$, I_R i I_L – intensity of radiation at the output of filters for horizontal, vertical, +45°, -45°, right and left circularly polarized components, corresponding by ($I_{+45°}$ and $I_{-45°}$ – intensities of radiation, transmitted across the linear polarizer, oriented along axis of polarizer at +45° and -45°).

Taking into account, that the above-mentioned values are interconnected by the relation

$$I_H + I_V = I_{+45^\circ} + I_{-45^\circ} = I_R + I_L = I\,, \tag{2}$$

Stocks vector of optic radiation can be determined by means of measuring the intensity of any independent light fluxes from above-mentioned six, for example by I_H, I_V, $I_{+45°}$ and I_L [2].

In such case Stocks vector

$$\boldsymbol{S} = \begin{bmatrix} I_H + I_V \\ I_H - I_V \\ 2I_{+45^\circ} - (I_H + I_V) \\ 2I_R - (I_H + I_V) \end{bmatrix}. \tag{3}$$

Application of Stocks vector provides the possibility to represent partially polarized radiation, for which the relation is performed

$$S_0^2 > S_1^2 + S_2^2 + S_3^2\,. \tag{4}$$

Partially polarized radiation can be decomposed into two components: completely polarized S_p and non-polarized S_{np}, and each Stocks parameter of output beam S equals the sum of corresponding parameters of this components

$$\begin{gathered} S = S_p + S_{np}; \\ S_{np} = \left\{\left[S_0 - \left(S_1^2 + S_2^2 + S_3^2\right)^{1/2}\right], 0, 0, 0\right\}; \\ S_p = \left\{\left(S_1^2 + S_2^2 + S_3^2\right)^{1/2}, S_1, S_2, S_3\right\}. \end{gathered} \tag{5}$$

For connection between the polarization states of radiation before and after its interaction with studied medium Mueller matrix 4x4 is used, its describes the transformation of radiation by the medium with deliberate polarization state, determined by the wavelength and for the preset direction of propagation [2]

$$\mathbf{M} = \begin{bmatrix} M_{0,0} & M_{0,1} & M_{0,2} & M_{0,3} \\ M_{1,0} & M_{1,1} & M_{1,2} & M_{1,3} \\ M_{2,0} & M_{2,1} & M_{2,2} & M_{2,3} \\ M_{3,0} & M_{3,1} & M_{3,2} & M_{3,3} \end{bmatrix}. \tag{6}$$

Elements of matrix **M** are valid and in general case independent numbers. For greater part of polarization elements (circular and linear polarizers, depolarizers, linear and circular deattenuators) Mueller table matrices are presented in literature [10].

Mueller matrix of the sample transforms Stocks vector of incident beam into corresponding Stocks vector of the output radiation

$$\mathbf{S}' = \mathbf{M} \cdot \mathbf{S}\,, \tag{7}$$

where $\mathbf{S}$, $\mathbf{S}'$ – Stocks vectors before and after interaction of radiation with the medium.

In general case 16 independent measurements must be carried out for determination of 4x4 Mueller matrix. Here we can apply normalized Stocks vectors for four states of polarization of incident beam H, V, +45° and R, which have the form

$$\boldsymbol{S}_H = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}, \ \boldsymbol{S}_V = \begin{pmatrix} 1 \\ -1 \\ 0 \\ 0 \end{pmatrix}, \ \boldsymbol{S}_{+45^\circ} = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \end{pmatrix}, \ \boldsymbol{S}_R = \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \tag{8}$$

where indices H, V, +45° and R represent states of horizontal and vertical linear polarization, +45° linear polarization and right circular, correspondingly.

Let us represent 4x4 Mueller matrix as the product of four vectors-columns, each having 4 elements

$$\mathbf{M} = \left[\mathbf{V_1} \cdot \mathbf{V_2} \cdot \mathbf{V_3} \cdot \mathbf{V_4}\right], \tag{9}$$

The corresponding normalized Stocks vectors of output radiation are experimentally determined by (8) for same polarization states of input radiation

$$\begin{cases} \mathbf{S}'_H = \mathbf{M} \cdot \mathbf{S}_H = \mathbf{V_1} + \mathbf{V}_2 \\ \mathbf{S}'_V = \mathbf{M} \cdot \mathbf{S}_V = \mathbf{V_1} - \mathbf{V_2} \\ \mathbf{S}'_{+45^\circ} = \mathbf{M} \cdot \mathbf{S}_{+45^\circ} = \mathbf{V_1} + \mathbf{V_3} \\ \mathbf{S}'_R = \mathbf{M} \cdot \mathbf{S}_R = \mathbf{V_1} + \mathbf{V_4} \end{cases}. \tag{10}$$

Thus, Mueller matrix of the sample can be calculated from four output Stocks vectors [2]

$$\mathbf{M} = \frac{1}{2} \times \left[\mathbf{S}'_H + \mathbf{S}'_V, \mathbf{S}'_H - \mathbf{S}'_V, 2\mathbf{S}'_{+45^\circ} - \left(\mathbf{S}'_H + \mathbf{S}'_V\right), 2\mathbf{S}'_R - \left(\mathbf{S}'_H + \mathbf{S}'_V\right)\right]. \tag{11}$$

For analysis and control of optical properties of biological tissues the basis, suggested by Marienko and Savenkov [15] is used, where anisotropic properties of the object are represented by sequential action of four main kinds of anisotropy: circular phase, linear phase, linear amplitude and circular amplitude anisotropy. Such sequential action is determined by following product [11]

$$\mathbf{M} = [\mathbf{M_{CP}}(\varphi)][\mathbf{M_{LP}}(\delta, \alpha)][\mathbf{M_{LA}}(P, \theta)][\mathbf{M_{CA}}(R)]\,. \tag{12}$$

The form of corresponding Mueller matrices of product components (12) is presented in literature [8,11].

Thus, general matrix $\mathbf{M}$ of the object (12) is the function of the following six parameters of anisotropy – R (transmission factor of the object with circular amplitude anisotropy), θ (orientation angle of partial linear polarizer), P (value of relative transmission of linear partial polarization), α (orientation of element with linear phase anisotropy), δ (phase shift, introduced by the element of the basic $[\mathbf{M_{LP}}]$) and ϕ (angle of relation of polarization azimuth to which the element with circular phase anisotropy $[\mathbf{M_{CP}}]$ leads). In carrying out of research, represented by the given Mueller matrix $\mathbf{M}$, the dominant determined anisotropic mechanism can be allocated [12].

3. AUTOMATIC IMAGING POLARIMETER

Block-diagram of the experimental installation, developed in cooperation with S.N Savenkov, A.S Klimov and Ye.A. Oberemok and which is used in the given research is presented in Fig. 1. He-Ne laser (Spectra Physics) with the central wavelength λ=0,6328 μm and maximum power of 50 mW is used as the source of radiation. Laser radiation is reflected from rotating mirrors and enters across coherency scrambler the collimator that forms wide beam of parallel rays with flat wave front. The formation of polarization state in the channel I of polarimeter occurs by means of opto-mechanical modulator, it consists of retarder 1 and polarizer. These components can rotate around the axis by means of drives from corresponding step motors. At the output of opto-mechanical modulator linearly polarized wave with required azimuth of polarization relatively the plane of incidence is formed. Field of laser radiation, scattered by the sample of biotissue, passes across receiving channel II of polarimeter, that consists of retarder 2, analyzer, lens by means of which the image is protected in the plane of registration of CCD-matrix, and digital CCD camera KPC-301CZH (KT&C). Calibration of the chamber is carried out by means of the program using the obtained experimentally calibrated characteristic (as the standard photodiode with linear transmitting characteristic is used). The chamber is connected electrically across frame grabber with personal computer (PC). Control and synchronization of retarder's position, connected with windings of step motors is realized by means of special block of microcontroller (CPU), it is also connected to PC. Calibration and control of the elements of polarimeter, measuring and control of Stocks vector parameters and anisotropy as well as reconstruction, storage and visualization of images both of complete (4x4) and non-complete (4x3) Mueller matrices [9] depending on forming circuit of modulator occurs by means of special computer program Polyset.

Characteristic feature of device operation while performing investigation of biotissue samples is the possibility to carry out automatic measurements and visualization of complete and non-complete (4x3) Mueller matrices applying the method of three probing polarization [13,14], that enables to improve considerably (up to 30%) the accuracy of measurements results and decrease by 25% time, needed for data processing [13]. It allows to enlarge functional possibilities of laser non-destructive diagnosis and control of optical parameters of anisotropic objects and media, in particular, heterogeneous biotissues well as optimize investigations dealing with dynamics of measuring parameters in cross-section of image frame, depending on the sample. For *in vivo* research measurements are carried out in reflected light, forming and receiving channel are located in the same way as ellipsometers [7] at determined angles (approximately 20 ° to normal), this minimizes the influence Fresnel reflection.

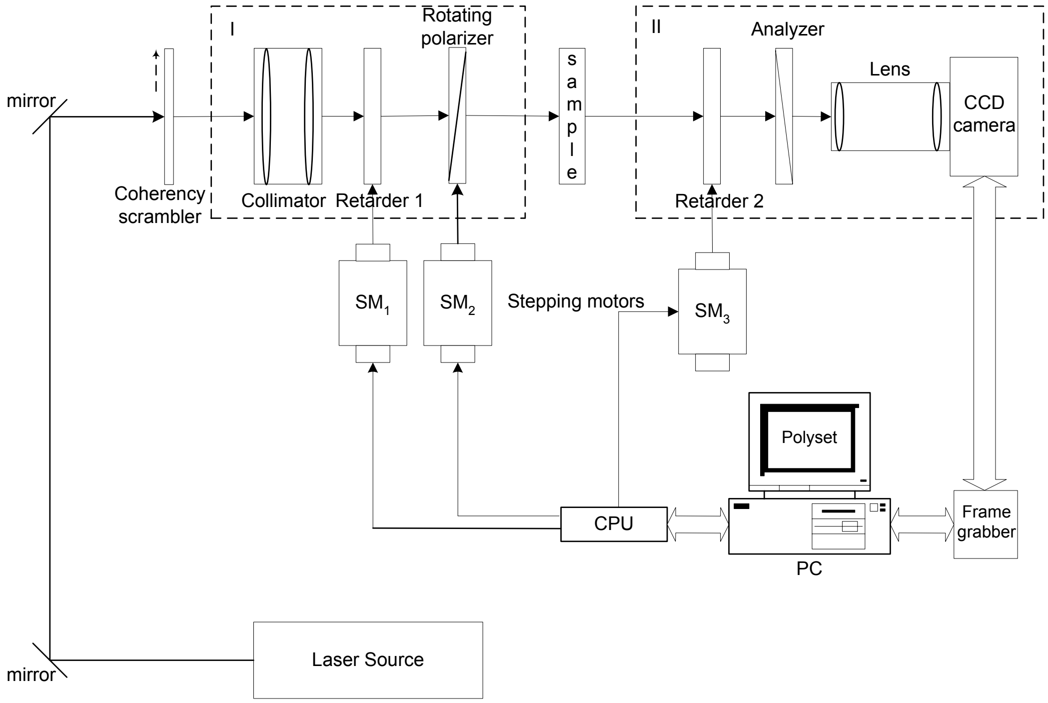


Fig. 1. Block-diagram of automatic laser-based imaging polarimeter

4. EXPERIMENTAL RESULTS AND DISCUSSION

In tha process of investigation thin flat section were used (thickness is approximately 50 μm) fixed in Canadian embalm and dermis of frozen human skin with tumors (melanoma) and without them. Measurements were carried out in the mode of direct passage of light applying the method of polarization microscopy [2,8]. The microscope was placed between retarder 2and analyzer of receiving channel II of imaging polarimeter. After measurement of Stocks vectors parameters and computation of corresponding experimental Mueller matrices as a result of possible depolarization of radiation by investigated object their preliminary processing was carried out applying Claude procedure [15]. To allocate non-depolarizing part of obtained Mueller matrices the method of matrix coherency was used [16]. The given method determines connection of Mueller matrices elements **M** of investigated object with matrix of coherency **T** in the following way

$$\begin{aligned}
&T_{0,0}=\frac{1}{4}\left(M_{0,0}+M_{1,1}+M_{2,2}+M_{3,3}\right); \quad T_{0,3}=\frac{1}{4}\left(M_{0,3}-iM_{1,2}+iM_{2,1}+M_{3,0}\right);\\
&T_{1,1}=\frac{1}{4}\left(M_{0,0}+M_{1,1}-M_{2,2}-M_{3,3}\right); \quad T_{1,2}=\frac{1}{4}\left(iM_{0,3}+M_{1,2}+M_{2,1}-iM_{3,0}\right);\\
&T_{2,2}=\frac{1}{4}\left(M_{0,0}-M_{1,1}+M_{2,2}-M_{3,3}\right); \quad T_{2,1}=\frac{1}{4}\left(-iM_{0,3}+M_{1,2}+M_{2,1}+iM_{3,0}\right);\\
&T_{3,3}=\frac{1}{4}\left(M_{0,0}-M_{1,1}-M_{2,2}+M_{3,3}\right); \quad T_{3,0}=\frac{1}{4}\left(M_{0,3}+iM_{1,2}-iM_{2,1}+M_{3,0}\right).
\end{aligned} \tag{13}$$

$$\begin{aligned}
&T_{0,1}=\frac{1}{4}\left(M_{0,1}+M_{1,0}-iM_{2,3}+iM_{3,2}\right); \quad T_{0,2}=\frac{1}{4}\left(M_{0,2}+M_{2,0}+iM_{1,3}-iM_{3,1}\right);\\
&T_{1,0}=\frac{1}{4}\left(M_{0,1}+M_{1,0}+iM_{2,3}-iM_{3,2}\right); \quad T_{2,0}=\frac{1}{4}\left(M_{0,2}+M_{2,0}-iM_{1,3}+iM_{3,1}\right);\\
&T_{2,3}=\frac{1}{4}\left(iM_{0,1}-iM_{1,0}+M_{2,3}+M_{3,2}\right); \quad T_{1,3}=\frac{1}{4}\left(-iM_{0,2}+iM_{2,0}+M_{1,3}+M_{3,1}\right);\\
&T_{3,2}=\frac{1}{4}\left(-iM_{0,1}+iM_{1,0}+M_{2,3}+M_{3,2}\right); \quad T_{3,1}=\frac{1}{4}\left(iM_{0,2}-iM_{2,0}+M_{1,3}+M_{3,1}\right).
\end{aligned} \tag{14}$$

Elements of T matrix are linearly independent. Matrix always has four valid proper numbers $\mu_{1,2,3,4}$ as **T** is hermitian ($T_{i,j} = T^{*}_{i,j}$). Proper vectors of coherency matrix $\mathbf{\Psi}^{1,2,3,4}$ are Jones matrices $\mathbf{J}^{1,2,3,4}$ written in column according to the following rule

$$\mathbf{J}^{\mathrm{k}}_{0,0} = \Psi^{k}_{0} + \Psi^{k}_{1}, \quad \mathbf{J}^{k}_{0,1} = \Psi^{k}_{2} - i\Psi^{k}_{3}, \quad \mathbf{J}^{k}_{1,0} = \Psi^{k}_{2} + i\Psi^{k}_{3}, \quad \mathbf{J}^{k}_{1,1} = \Psi^{k}_{0} - \Psi^{k}_{1}; \quad k = \overline{1,4}, \tag{15}$$

where k – number of proper vector of coherency matrix.

Output Mueller matrix is represented by the sum of four determined (nondepolarizing) Mueller matrices $\mathbf{M}^{1,2,3,4}_{D}$ calculated from Jones matrices [16]. Proper numbers of coherency matrix play the role of weight multipliers at corresponding adders in the sum:

$$\mathbf{M} = \sum_{k=1}^{4} \mu_k \mathbf{M}^{k}_{D}; \qquad \mathbf{M}^{k}_{D} \Leftrightarrow \mathbf{J}^{k}. \tag{16}$$

Thus, anisotropic properties of the object are represented simultaneously by the parallel action of four determined anisotropic mechanisms. If three out of four proper numbers of coherency matrix have zero value, then corresponding Mueller matrix is determined object. If all four proper numbers of coherency matrix differ from zero, then it is thought [15] that in the given object anisotropic mechanism prevails, which describes Mueller matrix near maximum weight multiplier in the sum (14). In case of negative proper numbers output Mueller matrix can't be physically realized within linear interaction of radiation with the sample.

In case of small depolarization one of proper numbers considerably exceeds three others determined properties of the object can be studied on the base of analysis of prevailing determined adder in the sum (16). Criterion for evaluation of the possibility of allocation from the given object the prevailing determined part is the value of object entropy [12]. Object entropy H is calculated on the base of coherency matrix of proper numbers as

$$H = -\sum_{r=1}^{N} K_r \log_N \left(K_r\right); \qquad K_r = \frac{\mu_r}{\sum_{j=1}^{4} \mu_j}, \tag{17}$$

where N is selected if $0 \le H \le 1$, in case of positiveness of all proper numbers and small (within the limit of the error) [12] negative proper numbers $N = 4$.

Entropy characterizes the degree of anisotropic decomposition of the object, that leads to depolarization of polarized radiation. It is thought, that in the object, represented by Mueller matrix **M**, we can allocate prevailing determined anisotropic mechanism, if value of entropy is $H \le 0.5$.

The procedure of Mueller matrices determination described above, together with other mathematical operations is realized in software package Mathematica®.

Experimental Mueller matrices for the thin sections of human skin dermis (size of image 340x340), which demonstrate the results of program operation are shown in Fig. 2, 3.

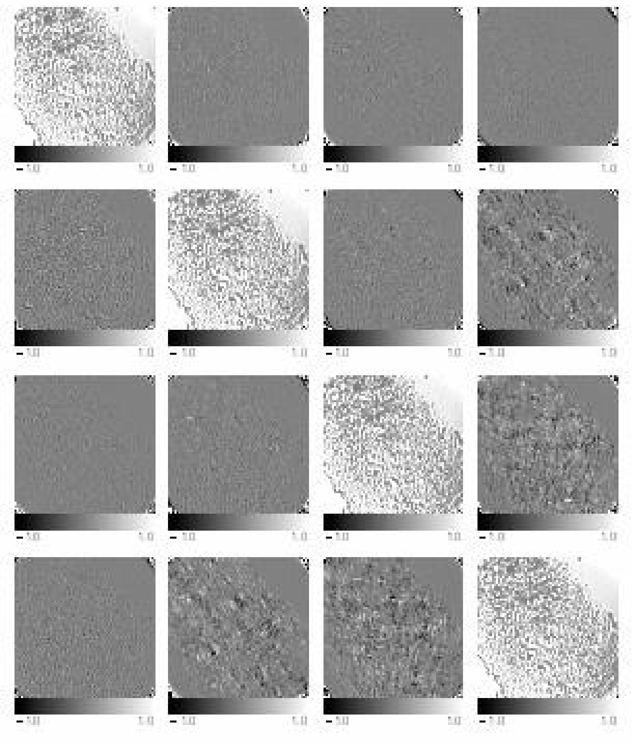


Fig. 2. Mueller matrix of dermis section 1 (without processing)

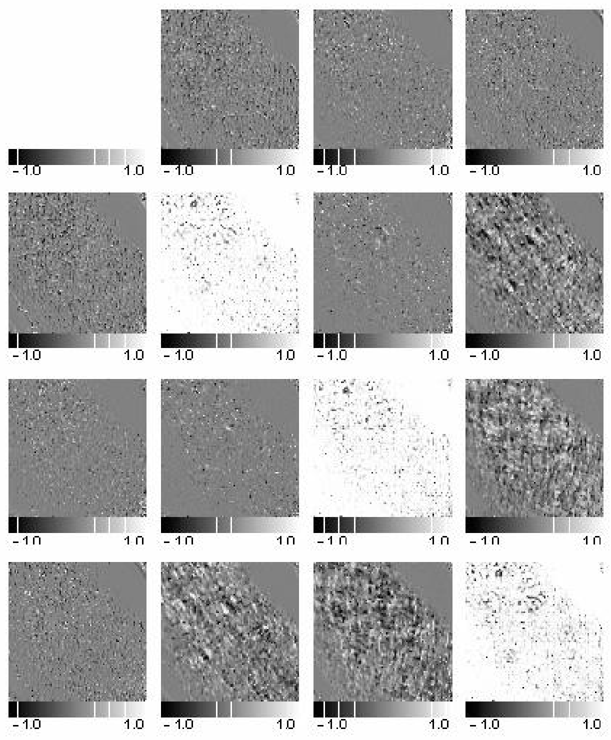


Fig.3. Mueller matrix of dermis section 1 (with processing)

Fig. 4, 5 show experimental Mueller matrices of human skin dermis section (image 340x340) for samples with areas of pathological changes as a result of melanoma 3 (Fig. 5) and without changes 2 (Fig. 4). For undamaged areas of dermis among known anisotropic mechanisms birefringence is related, value δ changes in accordance with thickness and density of tissue fibers. In the same way smoothly along the same direction of fibers axis orientation of birefringence α changes too (Fig. 6). In Fig. 7 the density of α, δ and H parameters distribution for sample 2 is presented. In Fig. 8 the results of vector analysis of parameters of linear phase anisotropy α and δ for sample 2 are demonstrated (the output matrix of images 340x340 is converted in matrix of 40x40 with corresponding averaging of parameters by squares). The length of arrows in Fig. 8 corresponds to the value of δ parameter, and their direction corresponds to the direction of α orientation according to Fig. 6.

For damaged areas of tissue in sample 3 the value of birefringence is more homogeneous in the plane of cross-section, and in the area with visible damage (presented by dotted line in Fig. 8) they are practically missing. That is why orientation of α in damaged areas is of chaotic character. The same processes were observed also for services of other samples of skin epidermis and dermis (5 samples with melanoma and 5 samples without).

Fig. 9 shows density of α, δ and H parameters for sample 3.

Thus can make a conclusion, that melanoma damages cells of epidermis and skin dermis and as a result they loose their anisotropic properties (become practically isotropic) and partially depolarize radiation. The nature of these phenomena requires further investigation. At the same time orientation order is a characteristic feature for sections of undamaged skin.

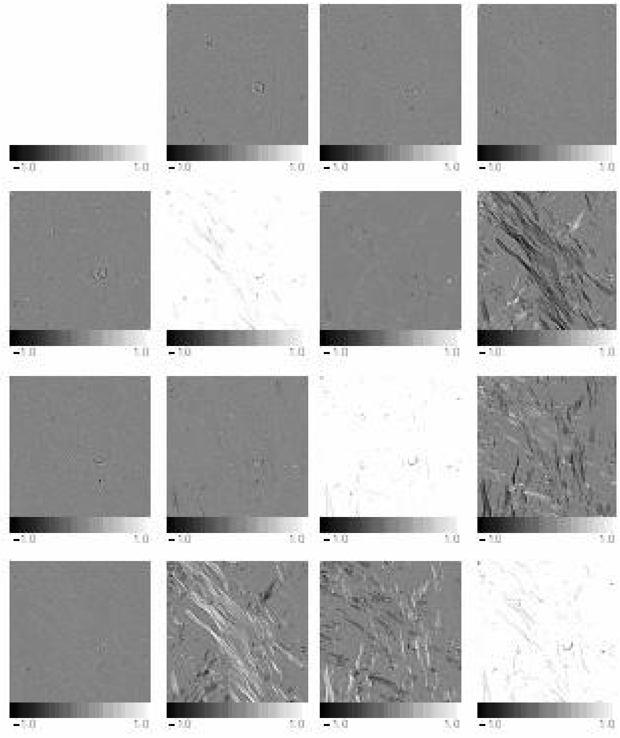


Fig. 4. Mueller matrix of skin dermis (sample 2)

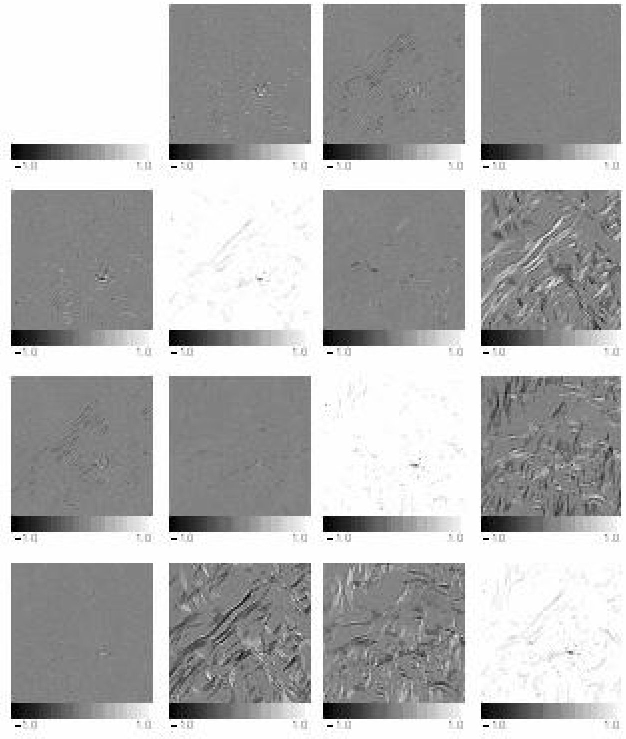
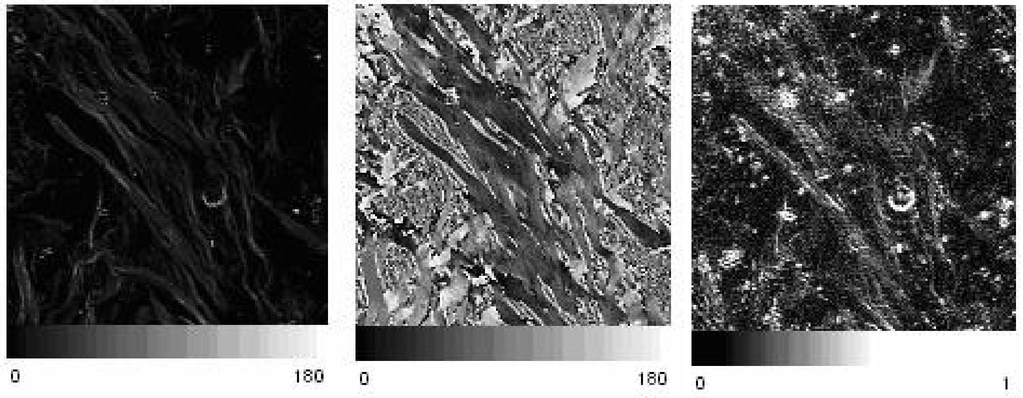
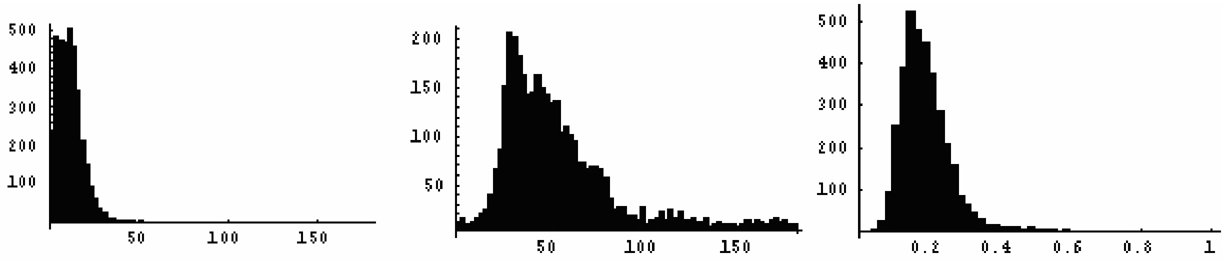


Fig. 5. Mueller matrix of skin dermis (sample 3 with melanoma)



α (deg.) *δ* (deg.) *H*

Fig. 6. Polarization parameters *α*, *δ* and *H* for sample 2



α (deg.) *δ* (deg.) *H*

Fig.7. Density of *α*, *δ* and *H* parameter distribution (in conventional units) for sample 2

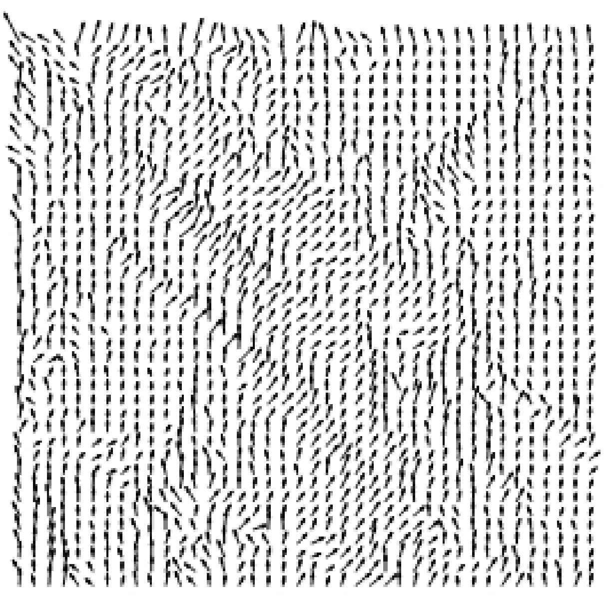
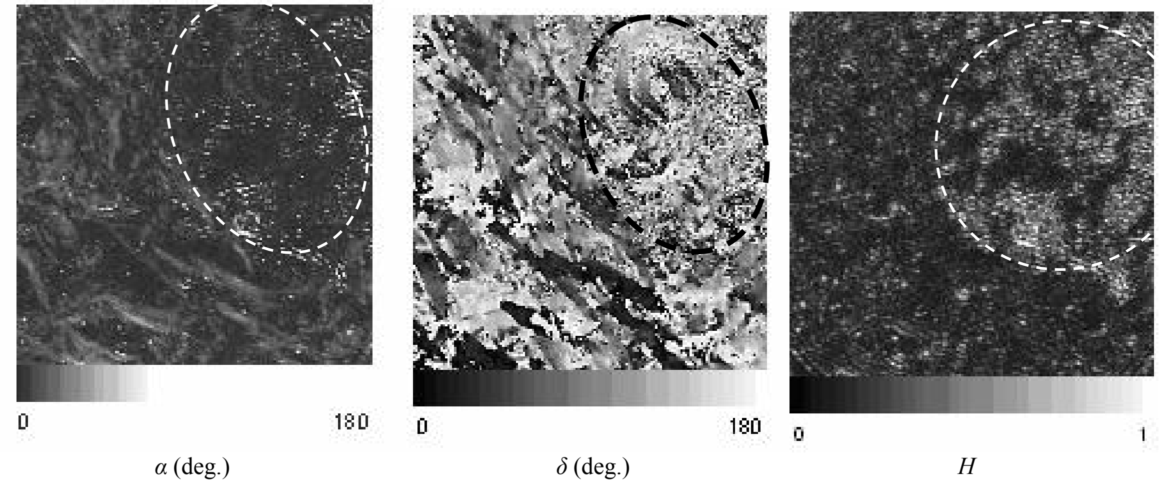
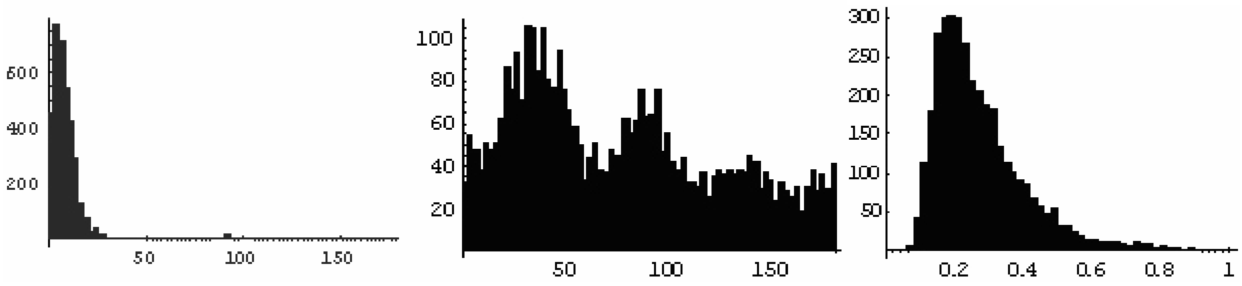


Fig. 8. Vector graphic of anisotropy parameters *α* and *δ* distribution for sample 2



α (deg.) *δ* (deg.) *H*

Fig. 9. Polarization parameters *α*, *δ* and *H* for sample 3 (dotted line shows the area of visible damage by melanoma)



α (deg.) *δ* (deg.) *H*

Fig.10. Density of parameters *α*, *δ* and *H* distribution (in conventional units) for sample 3

5. CONCLUSIONS

The paper present the possibility of engineering and techniques of automated imaging polarimetry for experimental investigation of thin sections heterogeneous by cross-section of biotissues with anisotropic properties. The connection between polarization characteristics of flat se thin sections of human skin dermis and pathological changes due to melanoma is analyzed. The suggested approach can be applied for study of polarization parameters of heterogeneous scattered anisotropic media of any origin (to reveal stresses, temperature gradients in crystal optics etc.).

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