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Azimuthally stable laser polarimetry of polycrystalline films of human biological fluids

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ABSTRACT

The model of Mueller-matrix description of mechanisms of optical anisotropy typical for polycrystalline films of liquor - optical activity, birefringence, as well as linear and circular dichroism - is suggested. Within the statistical analysis of such distributions the objective criteria of differentiation of films of liquor from the dead you people different times were determined. From the point of view of probative medicine the operational characteristics (sensitivity, specificity and accuracy) of the method of Mueller-matrix reconstruction of optical anisotropy parameters were found and its efficiency in another task - diagnostics of diseases of internal organs of rats was demonstrated.

Keywords: polarimetry, liquor, laser image, biological fluids.

1. INTRODUCTION

Biological tissues and fluids represent structurally inhomogeneous media with absorption. To describe interactions of polarized light with such complex systems the most general approaches based on Mueller-matrix formalism are required. Nowadays in biological and medical investigations many practical techniques based on measurement and analysis of Mueller matrices of investigated samples are used [1-12]. In recent 10-15 years a separate approach – laser polarimetry [13] – was formed in matrix optics. On its basis the interactions between the set of statistical moments of the 1st-4th orders characterizing Mueller-matrix elements distribution and parameters of linear birefringence of fibrillar protein networks of human biological tissues were determined. This enabled to diagnose oncological changes of skin derma, epithelial and connective tissue of women's reproductive sphere organs, etc. [18-21]. In addition, laser polarimetry techniques require further development and generalization.

Firstly, not all elements of Mueller matrix prove to be convenient for characterizing biological samples. The reason of this is the azimuthal dependence of the majority of matrix elements – generally 12 of 16 elements change at rotation of the sample around the probing axis.

Secondly, the spectrum of mechanisms of optical anisotropy of biological layers is not confined to linear birefringence only. Taking into consideration the impact of other mechanisms – circular birefringence, as well as linear and circular dichroism – is topical in the aspect of enlarging the range of diagnostic techniques.

Thirdly, there is a wide range of optically anisotropic biological objects, for which laser polarimetry techniques did not spread widely. Biological fluids – blood and its plasma, urine, bile, saliva and others – belong to them. The objects of this class are easily accessible and do not require the traumatic surgery of biopsy.

This research is focused on generalization of optical anisotropy of optically thin layers of liquor films and histological sections of the internal organs of healthy and diabetic rats and the development of the method of "azimuthally stable" Mueller-matrix reconstruction of anisotropy parameters of polycrystalline networks.

2. BRIEF THEORETICAL BACKGROUND

The basis of our work are based on modeling representations of phase anisotropy (optical activity and linear birefringence) of the polycrystalline structure of the films of blood plasma. In this approximation, the experimentally measured matrices have the following symmetry [22-28]

$$\{F\} = \{\Omega\}\{D\} = f_{11}^{-1} \times \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & f_{22} & f_{23} & f_{24} \\ 0 & f_{32} & f_{33} & f_{34} \\ 0 & f_{42} & f_{43} & f_{44} \end{vmatrix}. \quad (1)$$

Here $\{\Omega\}$ - Mueller matrix circular birefringence or optical activity of amino acid molecules

$$\{\Omega\} = \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & \omega_{22} & \omega_{23} & 0 \\ 0 & \omega_{32} & \omega_{33} & 0 \\ 0 & 0 & 0 & 1 \end{vmatrix}, \quad \omega_{ik} = \begin{cases} \omega_{22} = \omega_{33} = \cos 2\theta; \\ \omega_{23} = -\omega_{32} = \sin 2\theta. \end{cases} \quad (2)$$

where θ - rotation angle of polarization plane of the light beam transformed by amino acids.

Linear birefringence of amino acids polypeptide chains characterized by Mueller matrix $\{D\}$ of the following form

$$\{D\} = \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & d_{22} & d_{23} & d_{24} \\ 0 & d_{32} & d_{33} & d_{34} \\ 0 & d_{42} & d_{43} & d_{44} \end{vmatrix}, \quad d_{ik} = \begin{cases} d_{22} = \cos^2 2\rho + \sin^2 2\rho \cos \delta; \\ d_{23} = d_{32} = \cos 2\rho \sin 2\rho (1 - \cos \delta); \\ d_{33} = \sin^2 2\rho + \cos^2 2\rho \cos \delta; \\ d_{42} = -d_{24} = \sin 2\rho \sin \delta; \\ d_{34} = -d_{43} = \cos 2\rho \sin \delta; \\ d_{44} = \cos \delta. \end{cases} \quad (3)$$

Here ρ - direction of a optical axes; $\delta = \frac{2\pi}{\lambda} \Delta n l$ - phase shift between linearly polarized orthogonal components of light beam amplitude; λ - wavelength; Δn - birefringence; l - geometrical thickness.

$$\{M\} = \prod_{i=1}^4 \{M_i\} = M_{11}^{-1} \times \begin{vmatrix} 1 & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{vmatrix}. \quad (4)$$

Here $\{M_i\}$ - Mueller matrix that characterize the phase anisotropy ($\{\Omega\}, \{D\}$) and also amplitude anisotropy ($\{\Phi\}, \{\Psi\}$) amino acid molecules and their aggregates:

Circular dichroism

$$\{\Phi\} = \begin{vmatrix} 1 & 0 & 0 & \phi_{14} \\ 0 & \phi_{22} & 0 & 0 \\ 0 & 0 & \phi_{33} & 0 \\ \phi_{41} & 0 & 0 & 1 \end{vmatrix}, \quad \phi_{ik} = \begin{cases} \phi_{22} = \phi_{33} = \frac{1-C^2}{1+C^2}; \\ \phi_{14} = \phi_{41} = \pm \frac{2C}{1+C^2}. \end{cases} \quad (5)$$

Here $C = \frac{g_{\otimes} - g_{\oplus}}{g_{\otimes} + g_{\oplus}}$, g_{\otimes} , g_{\oplus} - absorption indices of left- (\otimes) and right-hand (\oplus) circularly polarized components

of light beam amplitude.

Linear dichroism

$$\{\Psi\} = \begin{vmatrix} 1 & \varphi_{12} & \varphi_{13} & 0 \\ \varphi_{21} & \varphi_{22} & \varphi_{23} & 0 \\ \varphi_{31} & \varphi_{32} & \varphi_{33} & 0 \\ 0 & 0 & 0 & \varphi_{44} \end{vmatrix}, \quad \varphi_{ik} = \begin{cases} \varphi_{12} = \varphi_{21} = (1 - \Delta\tau) \cos 2\rho; \\ \varphi_{13} = \varphi_{31} = (1 - \Delta\tau) \sin 2\rho; \\ \varphi_{22} = (1 + \Delta\tau) \cos^2 2\rho + 2\sqrt{\Delta\tau} \sin^2 2\rho; \\ \varphi_{23} = \varphi_{32} = (1 - \Delta\tau) \sin 2\rho; \\ \varphi_{33} = (1 + \Delta\tau) \sin^2 2\rho + 2\sqrt{\Delta\tau} \cos^2 2\rho; \\ \varphi_{44} = 2\sqrt{\Delta\tau}. \end{cases} \quad (6)$$

Here $\Delta\tau = \frac{\tau_x}{\tau_y}$, $\begin{cases} \tau_x \cong \tau \cos \rho; \\ \tau_y = \tau \sin \rho \end{cases}$, τ_x, τ_y - absorption coefficients of linearly polarized orthogonal components of

light beam amplitude.

$$\{M\} = \{\Phi\}\{\Psi\}\{D\}\{\Omega\}. \quad (7)$$

For analytical and practical application (7) we used the data of investigations [1, 2, 4]. Here it is shown that the following elements of matrix $\{M\}$ as well as their combinations are azimuthally stable, independent of the sample rotation angle (Θ)

$$\begin{cases} M_{11}(\Theta) = \text{const}; M_{14}(\Theta) = \text{const}; \\ M_{41}(\Theta) = \text{const}; M_{44}(\Theta) = \text{const}; \end{cases} \begin{cases} [M_{22} + M_{33}](\Theta) \equiv \Sigma M_{22,33}(\Theta) = \text{const}; \\ [M_{23} - M_{32}](\Theta) \equiv \Delta M_{23,32}(\Theta) = \text{const}. \end{cases} \quad (8)$$

3. ANALYSIS AND DISCUSSION OF EXPERIMENTAL DATA

The measurements of coordinate distributions of Mueller-matrix elements (distribution of values in the film plane of blood plasma) were performed in the setup (Fig. 1) of the standard Stokes-polarimeter [13].

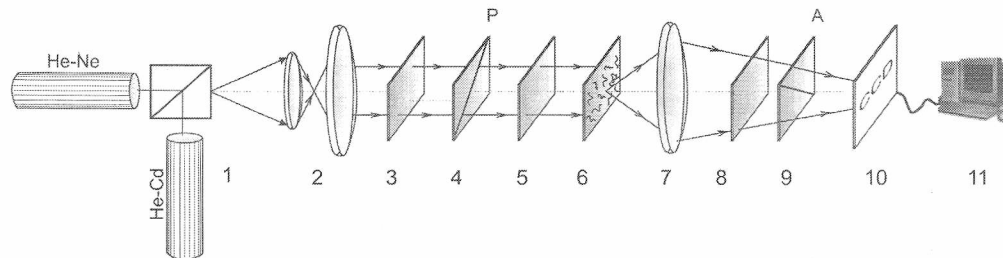


Fig. 1. Optical scheme of polarimeter, where 1 – He-Ne(Cd) laser; 2 – collimator; 3 – stationary quarter-wave plate; 5, 8 – mechanically movable quarter-wave plates; 4, 9 – polarizer and analyzer respectively; 6 – object of investigation; 7 – polarization microobjective; 10 – CCD camera; 11 – personal computer. Explanations are in the text.

Illumination of a sample under study was performed by the parallel ($\varnothing = 10^4$) laser beam of He-Ne ($\lambda = 0.6328 \mu\text{m}$, $W = 5.0 \text{ mW}$). The polarization light source consisted of quarter-wave plates 3; 5 and polarizer 4, it formed a right circularly polarized beam. Blood plasma films were placed in the focal plane of polarization microobjective 7 (focal distance – 30mm , aperture - 0.1, magnification – 4x). Behind the (Fourier) focal plane the vignetting diaphragm was located, its size changed within the range of 2 pix to 300 pix . Polarization microobjective 8 (focal distance – 30mm , aperture – 0.1, magnification – 4x) was located at the focal length form the frequency plane of lens 7 and, thus, performed inverse Fourier transform of a filtered out polarization field of laser radiation. The coordinate distribution of intensity of such fields, polarizationally filtered by quarter-wave plate 9 and polarizer 10, was registered in the plane of CCD-camera 11 (The Imaging Source DMK 41AU02.AS, monochrome 1/2" CCD, Sony ICX205AL (progressive scan); resolution – 1280×960 ; light sensitive area size – $7600 \times 6200 \mu\text{m}$; sensitivity – 0.05 lx; dynamic range – 8 bit; SNR – 9 bit, deviation of photosensitive characteristics from

linear no more than 15%). It provided the range of measuring the structural elements of polycrystalline network with the resolution of 2–2000 μm .

For the series of linearly (0^0 ; 45^0 ; 90^0) and right- (\otimes) circularly polarized probing laser beams (λ_1 and λ_2) the Stokes-vector parameters $S_{i=2,3,4}^{0;45;90;\otimes}$ were measured in the points of the digital image

$$\begin{cases} S_{i=2}^{0;45;90;\otimes}(\lambda_1, \lambda_2) = I_0^{0;45;90;\otimes} + I_{90}^{0;45;90;\otimes}; \\ S_{i=2}^{0;45;90;\otimes}(\lambda_1, \lambda_2) = I_0^{0;45;90;\otimes} - I_{90}^{0;45;90;\otimes}; \\ S_{i=3}^{0;45;90;\otimes}(\lambda_1, \lambda_2) = I_{45}^{0;45;90;\otimes} - I_{135}^{0;45;90;\otimes}; \\ S_{i=4}^{0;45;90;\otimes}(\lambda_1, \lambda_2) = I_{\otimes}^{0;45;90;\otimes} - I_{\oplus}^{0;45;90;\otimes}. \end{cases} \quad (9)$$

Here $I_{0;90;45;135;\otimes;\oplus}^{0;45;90;\otimes}$ - intensities of linearly ($0^0;90^0;45^0;135^0$), right- (\otimes) and left- (\oplus) circularly polarized components of the filtered (by means of polarizer 10 and quarter-wave plate 9) laser radiation.

Further the Mueller-matrix invariants were calculated (PC 10)

$$\begin{cases} M_{14}(\lambda_2) = S_1^{\otimes} - 0,5(S_1^0 + S_1^{90}); \\ M_{41}(\lambda_2) = 0,5(S_4^0 + S_4^{90}); \\ M_{44}(\lambda_1, \lambda_2) = S_4^{\otimes} - 0,5(S_4^0 + S_4^{90}); \\ \sum M_{22;33}(\lambda_1) = M_{22} + M_{33} = 0,5(S_2^0 - S_2^{90}) + S_3^{45} - 0,5(S_3^0 + S_3^{90}); \\ \Delta M_{23;32}(\lambda_1) = M_{23} - M_{32} = S_2^{45} - 0,5(S_2^0 + S_2^{90}) - 0,5(S_3^0 - S_3^{90}). \end{cases} \quad (10)$$

The series of Figures 2 -5 present the results of the technique of Mueller-matrix mapping of polycrystalline liquor films, taken over 1 hour and 3 hours after death.

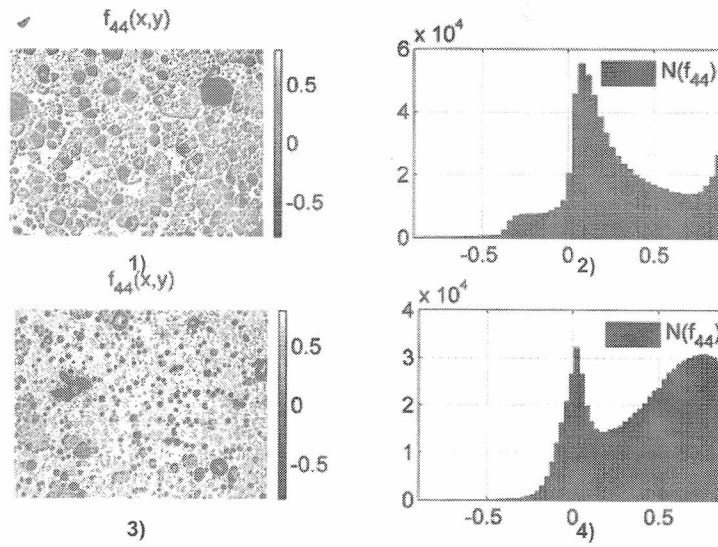


Fig. 2. Mueller-matrix image f_{44} taken by the film of liquor 1 hour ((1), (2)) and 3 hours ((3) and (4)) after death.

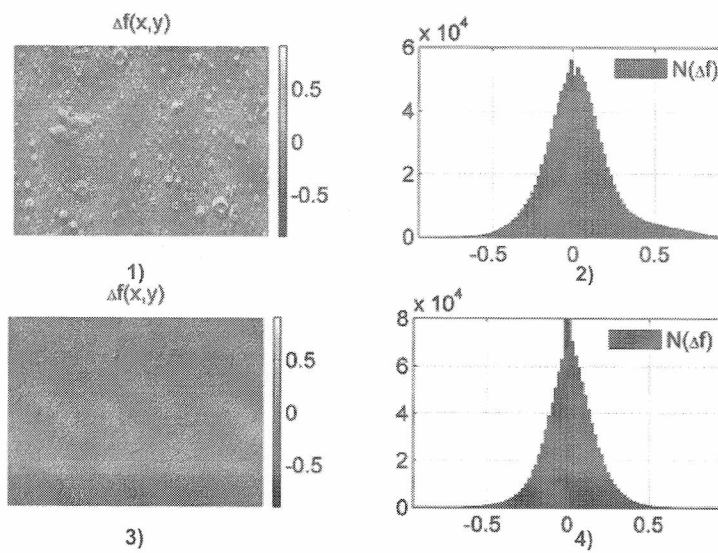


Fig. 3. Mueller-matrix image Δf taken by the film of liquor 1 hour ((1), (2)) and 3 hours ((3) and (4)) after death.

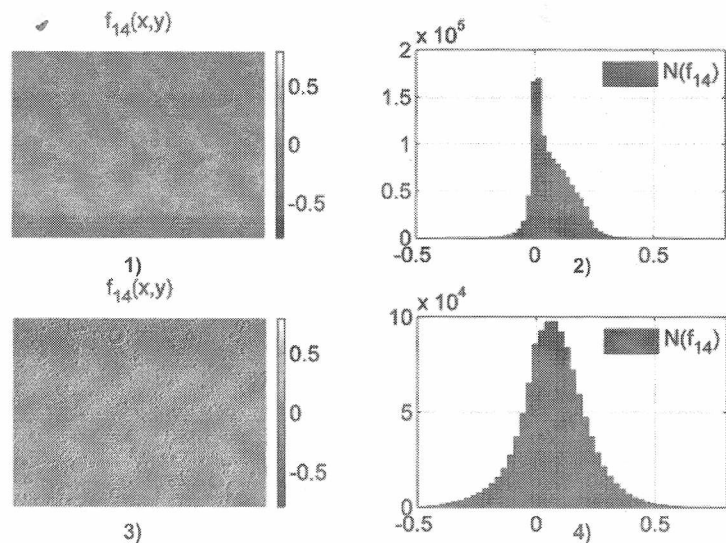


Fig. 4. Mueller-matrix image f_{14} taken by the film of liquor 1 hour ((1), (2)) and 3 hours ((3) and (4)) after death.

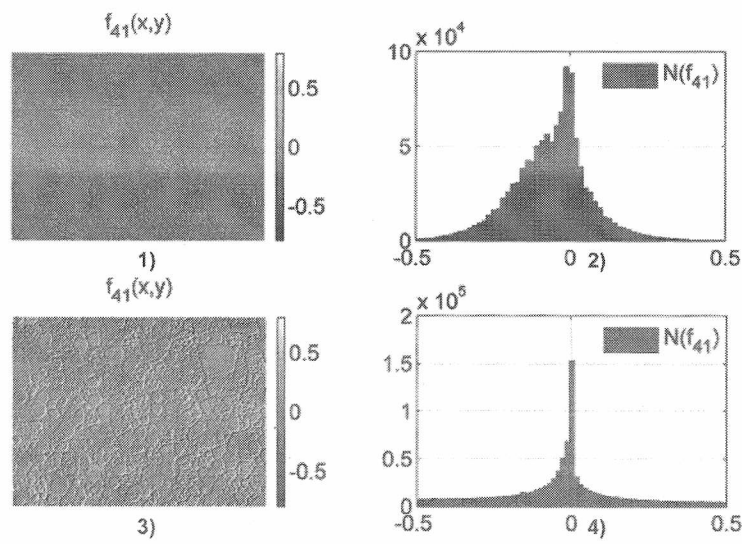


Fig. 5. Mueller-matrix image f_{41} taken by the film of liquor 1 hour ((1), (2)) and 3 hours ((3) and (4)) after death.

With a view to the possible use of Mueller-matrix method in determining the time of death for each group of samples were determined by the average (within group 1 and group 2) the magnitude of the statistical moments $\bar{Z}_{i=1,2,3,4}(q)$ and standard deviations $\pm \sigma$.

Following settings determine the accuracy of the time of death (ATD), the interval determine the accuracy of the method (Ω) and the ATD (ΔT).

Table 1. Timeframe and accuracy of the time of death

ATD, hours	ΔT	Ω
3	24	$\pm 0,1$
6	32	$\pm 0,15$
12	48	$\pm 0,2$

Table 2 illustrates the potential of this method to the other problem of Experimental Medicine - differentiation of histological sections of the internal organs of healthy and diabetic rats.

Table 2. Operational characteristics of the method of Mueller-matrix reconstruction

Parameters	Z_i	Kidney	Liver	Pancreas	Spleen
$Se(Z_i),\%$	Z_1	66,6%	71,9%	77,2%	77,2%
	Z_2	70,2%	70,2%	78,9%	82,4%
	Z_3	82,4%	85,9%	91,2%	92,9%
	Z_4	80,7%	89,4%	94,7%	94,7%
$Sp(Z_i),\%$	Z_1	63,1%	66,6%	73,6%	73,6%
	Z_2	66,6%	66,6%	75,4%	78,9%
	Z_3	80,7%	82,4%	87,8%	89,4%
	Z_4	77,2%	85,9%	91,2%	92,9%
$Ac(Z_i),\%$	Z_1	64,85%	69,25%	75,4%	75,4%
	Z_2	68,4%	68,4%	77,15%	80,65%
	Z_3	81,55%	84,15%	89,5%	91,15%
	Z_4	78,95%	87,65%	92,95%	93,8%

Here $Se(Z_i)$ - sensitivity, $Sp(Z_i)$ - specificity, $Ac(Z_i)$ - a diagnostic test accuracy; Z_i - the statistical moments of the 1st-4th orders that characterize the distribution of Mueller-matrix elements.

CONCLUSIONS

1. On the basis of the model of generalized optical anisotropy the two-wave technique of azimuthally invariant Mueller-matrix reconstruction of optical anisotropy parameters that are characteristic of polycrystalline liquor films was developed.

2. The Mueller-matrix invariants characterizing polarization manifestations of different (partial) mechanisms of optical anisotropy of protein networks were determined.
3. A method for determining the time of death based on the time of monitoring changes in the optical anisotropy of the polycrystalline films liquor was proposed.
4. The interrelations between the set of statistical moments of the 1st-4th orders characterizing the distributions of optical anisotropy parameters and the difference in polycrystalline structure of histological tissue sections of internal organs of rats were determined.
5. The effectiveness of the method of azimuthally invariant Mueller-matrix reconstruction of optical anisotropy parameters of blood plasma films in diagnostics of breast cancer was demonstrated.

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