

MUELLER MATRIX IMAGES 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 urine – optical activity and birefringence is suggested. On its basis the algorithms of reconstruction of parameters distribution (polarization plane rotations and phase shifts) of the indicated types of anisotropy were found. Within the statistical analysis of such distributions the objective criteria of differentiation of films of urine taken from healthy donors and albuminuria patients 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 diagnostics of albuminuria was demonstrated.

Key words: polarization, Mueller matrix, optical anisotropy, biological fluids, diagnostics.

1. INTRODUCTION

Biological tissues represent structurally inhomogeneous media. 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 [1–9]. In recent 10–15 years a separate approach – laser polarimetry [10] – 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 human organs, etc. [11–13]. 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 is topical in the aspect of enlarging the range of diagnostic techniques [14–19].

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.

The possibility of polarization investigation of urine for early detection of albuminuria is considered in this research. It is an urgent task of diagnosis of various pathological conditions of human kidney. Currently, the traditional differential diagnosis of such conditions includes the following steps: analysis of the patient's complaints, the biochemical analysis of urine protein, renal ultrasound diagnostics, professional judgment. The final step or gold standard is the nephrobiopsy of kidney tissue [20–25]. This set of techniques confidently diagnose disease, which corresponds to a moderate and pronounced increase the content of protein in the urine of the patient [26–28]. Along with this, the unsolved task is the creation of objective, low-cost and express screening method of albuminuria diagnostics at early stages of kidney pathology close to the gold standard.

This research is focused on generalization of optical anisotropy of optically thin layers of urine films and the development of the method of “azimuthally stable” Mueller-matrix reconstruction of anisotropy parameters of polycrystalline networks in the task of albuminuria diagnostics.

2. BRIEF THEORETICAL BACKGROUND

Our research is based on model representations of phase anisotropy (optical activity and linear birefringence) of polycrystalline structure of biological liquids films developed in [11, 14–19, 29–34]. It was determined that in this proximity the experimentally measured matrices possess the following symmetry

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

Here $\{\Omega\}$ is the Mueller matrix of circular birefringence or optical activity of

amino acids molecules

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

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

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

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

Here ρ is the direction of an optical axis; $\delta = \frac{2\pi}{\lambda} \Delta n l$ – phase shift between linearly polarized orthogonal components of light beam amplitude; λ – wave length; Δn – birefringence value; l – geometrical thickness of the layer.

The analysis of the obtained data [29–34] was performed within the direct task – statistical processing of distribution of experimentally measured matrix elements $F_{ik}(\theta, \rho, \delta)$ with further differentiation of the samples of urine taken from healthy and albuminuria patients. At that the inverse task of polarization reconstruction of parameters of phase anisotropy was not considered.

Taking into consideration the generalization of the optical anisotropy model a number of questions arises of both methodological and applied nature. *The first question is* – in what approximation should the measured Mueller matrices be analyzed? *The second question:* in what sequence should the product of partial Mueller matrices be written? *The third question:* how can the inverse problem be adequately solved and the algorithms of anisotropy determination parameters be obtained? *The fourth question:* which of matrix elements should be analyzed? The following materials of this article will be devoted to answering these questions.

To analyze Mueller matrices (1) we used the model of optically anisotropic medium, in which two basic types of phase anisotropy exist [10]. According to this model the process of transformation of laser radiation polarization by urine can be

represented in the form of a sequence of the following mechanisms: “optical activity” and “linear birefringence” of the molecules of amino acids and their complexes.

For analytical and practical application (1) we used the data of investigations [1, 9]. Here it is shown that the following elements of matrix $\{F\}$ as well as their combinations are azimuthally stable, independent of the sample rotation angle (Θ)

$$\begin{cases} f_{11}(\Theta) = \text{const}; & [f_{22} + f_{33}](\Theta) \equiv \Sigma f_{22,33}(\Theta) = \text{const}; \\ f_{44}(\Theta) = \text{const}; & [f_{23} - f_{32}](\Theta) \equiv \Delta f_{23,32}(\Theta) = \text{const}. \end{cases} \quad (4)$$

It follows from (4) that for development of azimuthally stable method of Mueller-matrix reconstruction of optical anisotropy it is necessary to transform into other algorithms

$$\begin{cases} \theta = W(f_{44}, \Sigma f_{22,33}, \Delta f_{23,32}) \\ \delta = H(M_{44}, \Sigma f_{22,33}, \Delta f_{23,32}) \end{cases} \quad (5)$$

and to verify the solutions stability (5) while changing the position of partial matrices $\{F\}_i$ in (1). Let us consider this statement in detail.

The matrix of the generalized optical anisotropy (1) taking into account (2), (3) becomes as follows

$$\{F\} = \{D\}\{\Omega\} = \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & (d_{22}\omega_{22} + d_{23}\omega_{32}) & (d_{22}\omega_{23} + d_{23}\omega_{33}) & d_{24} \\ 0 & (d_{32}\omega_{22} + d_{33}\omega_{32}) & (d_{32}\omega_{23} + d_{33}\omega_{33}) & d_{34} \\ 0 & (d_{42}\omega_{22} + d_{43}\omega_{32}) & (d_{42}\omega_{23} + d_{43}\omega_{33}) & d_{44} \end{vmatrix}. \quad (6)$$

From (6) we obtain azimuthally invariant algorithms of polarization reconstruction of parameters characterizing the phase anisotropy of polycrystalline film of urine

$$\begin{cases} \delta = \arccos f_{44}; \\ \theta = 0.5 \arctan \frac{\Delta f_{23,32}}{\Sigma f_{22,33}}. \end{cases} \quad (7)$$

To check the solution (7) stability we have considered another sequence of partial matrix operators $\{D\}\{\Omega\} \rightarrow \{\Omega\}\{D\}$ in the product (6)

$$\{\tilde{F}\} = \{\Omega\}\{D\} = \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & (d_{22}\omega_{22} + d_{32}\omega_{23}) & (d_{23}\omega_{22} + d_{33}\omega_{23}) & (d_{24}\omega_{22} + d_{34}\omega_{23}) \\ 0 & (d_{22}\omega_{32} + d_{32}\omega_{33}) & (d_{23}\omega_{32} + d_{33}\omega_{33}) & (d_{24}\omega_{32} + d_{34}\omega_{33}) \\ 0 & d_{42} & d_{43} & d_{44} \end{vmatrix}. \quad (8)$$

It is easy to see that the solution of the system of equations (8) in relation to parameters of linear (3) and circular (2) birefringence gives the similar (7) result.

Thus the one-valuedness of azimuthally stable polarization reconstruction of phase anisotropy parameters can be stated.

3. ANALYSIS AND DISCUSSION OF EXPERIMENTAL DATA

In the experimental part of the research the following algorithm was used:

1. *Determination of statistically reliable representative selection of patients with the known (referent) diagnosis.* By means of software product Statmate for 95% confidence interval ($p < 0.05$) a reliable quantity of people in group 1 (donors) and group 2 (albuminuria – $< 3\mu\text{g}/\mu\text{mol}$) was determined – $n = 57$.

2. *Samples production.* The samples of optically thin films (attenuation factor $\tau < 0.1$) were formed under identical conditions by placing a drop of urine on optically homogeneous glass. The resulting film was dried at room temperature ($t = 22^\circ\text{C}$).

3. *Measuring the coordinate distributions of Mueller-matrix elements.* The measurements of coordinate distributions of Mueller-matrix elements (distributions of values in the plane of urine film) were performed in the setup (Fig. 1) of the standard Stokes-polarimeter [10].

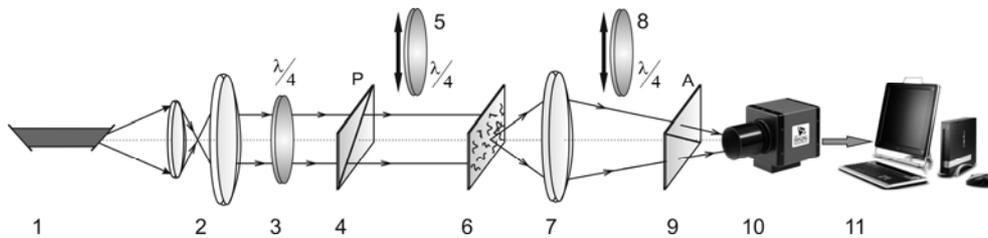


Fig. 1 – Optical scheme of polarimeter, where 1 – He-Ne laser; 2 – collimator; 3 – stationary quarter-wave plate; 5, 8 – mechanically movable quarter-wave plates; 4, 9 – polarizer and analyzer respectively; 6 – object of investigation; 7 – polarization microobjective; 10 – CCD camera; 11 – personal computer.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

Illumination of samples was performed by parallel ($\varnothing = 2 \times 10^3 \mu\text{m}$) weakly intensive ($W = 5 \text{ mW}$) beam 1 of He-Ne laser ($\lambda = 0.6328 \mu\text{m}$). Polarization light source consisted of quarterwave plate 3 and polarizer 4. The image of samples 6 were projected in the plane of light-sensitive plane of CCD-camera 10 (The Imaging Source DMK 41AU02.AS, monochrome 1/2" CCD, Sony ICX205AL (progressive scan); resolution – 1280×960 ; size of light-sensitive plate –

7600 × 6200 μm; sensitivity – 0.05 lx; dynamic range – 8 bit; SNR – 9 bit, deviation of photosensitive characteristics from the linear one does not exceed 15%) by means of microobjective 7 (Nikon CFI Achromat P, focal distance – 30 mm, NA – 0.1, magnification – 4×). Polarization analysis of the samples images was performed by means of quarterwave plate 8 and polarizer-analyzer 9.

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

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

Here $I_{0;90;45;135;\otimes;\oplus}^{0;45;90;\otimes}$ are the intensities of linearly (0°; 90°; 45°; 135°), right- (⊗) and left- (⊕) circularly polarized components of the filtered (by means of polarizer 9 and quarter-wave plate 8) laser radiation.

Further the Mueller-matrix invariants were calculated (PC 11)

$$\begin{cases} f_{44} = S_4^{\otimes} - 0.5(S_4^0 + S_4^{90}); \\ \sum f_{22,33} = f_{22} + f_{33} = 0.5(S_2^0 - S_2^{90}) + S_3^{45} - 0.5(S_3^0 + S_3^{90}); \\ \Delta f_{23,32} = f_{23} - f_{32} = S_2^{45} - 0.5(S_2^0 + S_2^{90}) - 0.5(S_3^0 - S_3^{90}). \end{cases} \quad (10)$$

4. *Polarization reconstruction of optical anisotropy parameters.* On the basis of (10) for each pixel of CCD-camera the (7) parameters of phase (δ, θ) anisotropy were found. For objective assessment of histograms $N(q)$ of distributions $q \equiv \{\delta, \theta\}$ the set of statistical moments of the 1st–4th orders was determined

$$\begin{aligned} Z_1 &= \frac{1}{P} \sum_{j=1}^P q_j; \quad Z_2 = \sqrt{\frac{1}{P} \sum_{j=1}^P (q_j)^2}; \\ Z_3 &= \frac{1}{Z_2^3} \frac{1}{P} \sum_{j=1}^P (q_j)^3; \quad Z_4 = \frac{1}{Z_2^4} \frac{1}{P} \sum_{j=1}^P (q_j)^4. \end{aligned} \quad (11)$$

Here P is the number of pixels of CCD-camera. These parameters characterize the mean (Z_1), dispersion (Z_2), skewness (Z_3) and kurtosis or “peak sharpness” (Z_4) of $N(q)$.

The series of Figs. 2–3 present the results of the technique of Mueller-matrix reconstruction parameters $q \equiv \{\delta, \theta\}$ of polycrystalline urine films. Each figure consists of the coordinate distributions (fragments (1), (3)) and the histograms $N(q)$ (fragments (2), (4)) for two arbitrary chosen samples of healthy (fragments (1), (2)) donors and those suffering from albuminuria patients (fragments (3), (4)).

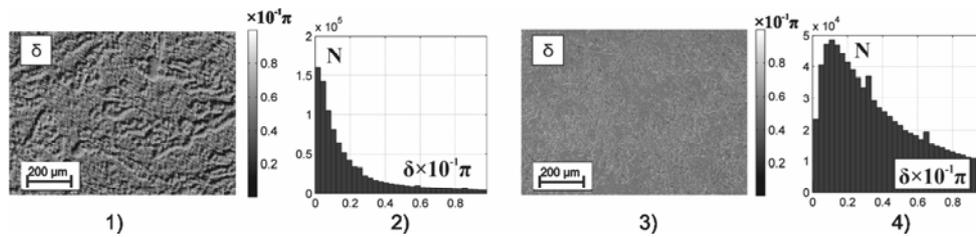


Fig. 2 – Coordinate distributions ((1), (3)) and the corresponding histograms ((2), (4)) of the values of phase shifts δ , formed by polycrystalline film of urine of donors ((1), (2)) and patients with albuminuria ((3), (4)).

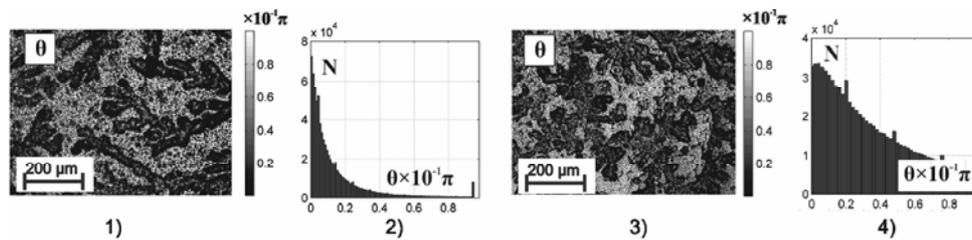


Fig. 3 – Coordinate distributions ((1), (3)) and the corresponding histograms ((2), (4)) of the values of phase shifts θ , formed by polycrystalline film of urine of donors ((1), (2)) and patients with albuminuria ((3), (4)).

The comparative analysis of the data obtained (Figs. 2–3) showed tendencies similar for the investigated samples:

Linear birefringence. It was determined that for the film of urine taken from a donor (Fig. 2, fragments (1)), the value of linear birefringence of lathlike globulin proteins is sufficiently less if compared to the sample (Fig. 2, fragments (3)) of albuminuria patients. The main extremes of histograms $N(\delta)$ of group 1 (Fig. 2, fragments (2)) are localized in the area of $\delta \rightarrow (0.07 \div 0.12) \times 10^{-1} \pi$. For group 2 (Fig. 2, fragments (4)) the bigger values of phase shifts – $\delta = (0.25 \div 0.35) \times 10^{-1} \pi$ are the most probable. Thus the increase of mean ($Z_1 \uparrow$) and dispersion ($Z_2 \uparrow$) of

histograms $N(\delta)$ appears to be a statistical indicator of albuminuria condition. At that the statistical moments of higher orders (skewness ($Z_3 \downarrow$) and kurtosis ($Z_4 \downarrow$)) decrease. Physically the obtained results can be related to the known data of biochemical analysis – greater concentration of albumin in the film of urine of albuminuria patient.

Circular birefringence. The similar tendencies, related to δ , in the statistical changes of parameter θ are determined (Fig. 3), characterizing the optical activity of globulin proteins (relations (2)) in urine films. Due to the increase of concentration of such proteins in urine of albuminuria patients the probability of greater values of θ , formed by the sample of group 2, increases (Fig. 3, fragments (3)). Quantitatively this illustrates the increase of probability of forming greater values $\theta = (0.4 \div 0.75) \times 10^{-1} \pi$ in distributions $N(\theta)$ (Fig. 3, fragments (4)). In other words, for oncological state the following statistical scenario is realized – $Z_1(\theta) \uparrow$; $Z_2(\theta) \uparrow$; $Z_3(\theta) \downarrow$; $Z_4(\theta) \downarrow$.

5. *Statistical intergroup analysis.* For the possible clinical application of both methods the following was determined for each group of samples [35–37]:

- average (within group 1 and group 2) values of statistical moments $\bar{Z}_{i=1;2;3;4}(q)$, their standard deviations $\pm \sigma$ and histograms $N(Z_i)$ – Table 1.
- traditional for probative medicine operational characteristics – sensitivity ($Se = \frac{a}{a+b} 100\%$), specificity ($Sp = \frac{c}{c+d} 100\%$) and balanced accuracy ($Ac = \frac{Se+Sp}{2}$), where a and b are the number of correct and wrong diagnoses within group 2; c and d – the same within group 1 – Table 2.

Differentiation of birefringence images of urine layers for each of polarizationally reconstructed parameters of phase anisotropy was performed by cross-sectional comparison of for distribution histograms $Z_{i=1;2;3;4}(q)$. If the mean value of this or that moment $\bar{Z}_i(q)$ in the test group 1 is not within the standard deviation σ of the investigated group 2, the difference between $\bar{Z}_i(q)$ is considered statistically reliable. Then the analysis of the region of overlap of histograms $Z_{i=1;2;3;4}(q)$ which determine the sensitivity Se , specificity Sp and accuracy Ac appears to be topical.

The comparative analysis of the data obtained (Table 1) showed that the differences between the values of average $\bar{Z}_{i=1;2;3;4}(q)$ moments of all orders are statistically reliable. However, there is an intergroup overlap for all histograms $N(Z_i)$. Moreover, the range of such an overlap is inversely proportional to the

value of the difference between the averages $\bar{Z}_{i=1;2;3;4}(q)$. The following quantitative differences between average statistical moments $\bar{Z}_i(q)$ are determined:

Linear birefringence – for Mueller-matrix reconstructed distributions δ of polycrystalline urine films of both types the difference between statistical moments $\bar{Z}_{i=1;2;3;4}(\delta)$ is $\{\Delta Z_1(\delta) = 1.43; \Delta Z_2(\delta) = 1.72; \Delta Z_3(\delta) = 1.78; \Delta Z_4(\delta) = 1.74\}$.

Table 1

Average ($\bar{Z}_{i=1;2;3;4}$) and standard deviations ($\pm \sigma$) of statistical moments $Z_{i=1;2;3;4}$ of optical anisotropy distributions of urine of groups 1 and 2

q	δ ($n = 57$)		θ ($n = 57$)	
	group 1	group 2	group 1	group 2
Z_1	$0,09 \pm 0,007$	$0,11 \pm 0,09$	$0,07 \pm 0,005$	$0,1 \pm 0,007$
Z_2	$0,14 \pm 0,011$	$0,21 \pm 0,016$	$0,11 \pm 0,008$	$0,19 \pm 0,015$
Z_3	$0,85 \pm 0,061$	$0,49 \pm 0,036$	$0,96 \pm 0,074$	$0,54 \pm 0,041$
Z_4	$1,14 \pm 0,083$	$0,65 \pm 0,047$	$1,34 \pm 0,01$	$0,77 \pm 0,057$

• *Circular birefringence* – for statistical moments $\bar{Z}_{i=1;2;3;4}(q)$ characterizing the distribution θ , formed by optically active structures of globulin of urine film are determined: $\{\Delta Z_1(\theta) = 1.22; \Delta Z_2(\theta) = 1.5; \Delta Z_3(\theta) = 1.73; \Delta Z_4(\theta) = 1.75\}$.

As the data presented show, the statistical moments of the 3rd and 4th orders characterizing the histograms $N(q)$ of the urine films of both groups of patients prove to be the most sensitive. On the other hand, the greater $\Delta Z_{i=1;2;3;4}(q)$ is, the more informative ($Se \uparrow$; $Sp \uparrow$; $Ac \uparrow$) the method appears to be.

Table 2

Operational characteristics of the method of Mueller-matrix reconstruction of polycrystalline structure of urine films

q	Z_i	δ	θ
$Ac(Z_i)$	Z_1	74%	78%
	Z_2	83%	87%
	Z_3	91%	90%
	Z_4	93%	95%

Table 2 presents the parameters of information value of azimuthally stable method of Mueller-matrix reconstruction of phase anisotropy of polycrystalline films of urine.

The comparative analysis of operational characteristics of the method of Mueller-matrix polarization reconstruction of polycrystalline structure of urine films revealed clinically optimal (highlighted in grey) parameters:

$$\begin{cases} \delta \rightarrow R(\delta) \equiv \{Ac(Z_{3;4}) = 91\% - 93\%\}; \\ \theta \rightarrow R(\theta) \equiv \{Ac(Z_{3;4}) = 90\% - 95\%\}. \end{cases} \quad (12)$$

The obtained results enable to state a rather high level of accuracy of azimuthally stable Mueller-matrix mapping. According to the criteria of probative medicine [32–34] the parameters $R(\delta, \theta) \sim 90\% - 95\%$ correspond to high quality.

6. *Comparative analysis with the methods of polarization and Mueller-matrix mapping.* The suggested method of Mueller-matrix polarization reconstruction shows several fundamental and applied advantages if compared with previously developed methods of biological liquids films polarimetry [29–34]:

- Fundamental advantages – all of mechanisms of phase anisotropy were analyzed. On this basis in the approximation of single scattering the algorithms of polarization reconstruction of optical anisotropy parameters possessed by polycrystalline urine films were found. That is why the information value of azimuthally stable Mueller-matrix mapping is considerably greater than that of the methods suggested in [29–34].

- Applied advantages – the results obtained using our method are reliable, reproducible and rather accurate ($Ac = 83\% - 95\%$) in comparison with azimuthally dependent methods of Mueller-matrix [29] and polarization mapping [30]. Azimuthally stable methods of Fourier phasometry [31–33] are less accurate ($Ac \leq 70\% - 75\%$) due to impossibility of complete spatial-frequency separation of the impact of the mechanisms of phase anisotropy. Moreover, the method of Fourier phasometry is rather sophisticated and requires precision adjustment of optical scheme by qualified technical personnel.

7. *Prospects of clinical application.* The suggested method can be used in mass screening of patients as an express-method. Still it is too early to claim that the investigation of this problem is completed. The fact is that other pathology can also express the “alarm” signals. Thus to implement this method into routine laboratory practice numerous clinical tests are required both in concomitant fields of medicine.

4. CONCLUSIONS

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

2. The Mueller-matrix invariants characterizing polarization manifestations of different (partial) mechanisms of optical anisotropy of biological liquids films polycrystalline networks were determined.

3. The interrelations between the set of statistical moments of the 1st–4th order characterizing the distributions of optical anisotropy parameters and the difference in polycrystalline structure of urine films of healthy people and albuminuria patients were determined.

4. The effectiveness of the method of azimuthally invariant Mueller-matrix reconstruction of optical anisotropy parameters of urine films in diagnostics of early stages of albuminuria was demonstrated.

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