

PROCEEDINGS OF SPIE

Biosensing and Nanomedicine VII

Hooman Mohseni
Massoud H. Agahi
Manijeh Razeghi
Editors

17–20 August 2014
San Diego, California, United States

Sponsored and Published by
SPIE

Volume 9166

Proceedings of SPIE 0277-786X, V. 9166

SPIE is an international society advancing an interdisciplinary approach to the science and application of light.

Biosensing and Nanomedicine VII, edited by Hooman Mohseni, Massoud H. Agahi, Manijeh Razeghi,
Proc. of SPIE Vol. 9166, 916601 · © 2014 SPIE · CCC code: 0277-786X/14/\$18 · doi: 10.1117/12.2081203

Proc. of SPIE Vol. 9166 916601-1

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Author(s), "Title of Paper," in *Biosensing and Nanomedicine VII*, edited by Hooman Mohseni, Massoud H. Agahi, Manijeh Razezghi, Proceedings of SPIE Vol. 9166 (SPIE, Bellingham, WA, 2014) Article CID Number.

ISSN: 0277-786X

ISBN: 9781628411935

Published by

SPIE

P.O. Box 10, Bellingham, Washington 98227-0010 USA

Telephone +1 360 676 3290 (Pacific Time) · Fax +1 360 647 1445

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Contents

v *Authors*
vii *Conference Committee*

SESSION 1 NANO DRUG DELIVERY

- 9166 04 **Incorporation of photosensitizer hypericin into synthetic lipid-based nano-particles for drug delivery and large unilamellar vesicles with different content of cholesterol** [9166-3]
- 9166 05 **Targeting hepatocellular carcinoma with aptamer-functionalized PLGA/PLA-PEG nanoparticles (Invited Paper)** [9166-4]
- 9166 06 **Nanotechnology-based treatment for chemotherapy-resistant breast cancer** [9166-5]
- 9166 07 **Feedback-mediated cancer therapy: a FRET-based nanoreporter approach** [9166-6]
- 9166 0A **Controlling the intracellular fate of nano-bioconjugates: pathways for realizing nanoparticle-mediated theranostics (Invited Paper)** [9166-10]

SESSION 2 BIOSENSING I

- 9166 0H **Label free detection of phospholipids by infrared absorption spectroscopy** [9166-17]

SESSION 3 BIOSENSING II

- 9166 0L **Electron optics of nanoplasmonic metamaterials in bio/opto theranostics (Invited Paper)** [9166-19]
- 9166 0M **Improving the performance of silicon photonic rings, disks, and Bragg gratings for use in label-free biosensing (Invited Paper)** [9166-20]
- 9166 0N **Novel 3D plasmonic nano-electrodes for cellular investigations and neural interfaces** [9166-21]
- 9166 0O **The whispering gallery mode biosensor: label-free detection from virus to single protein (Invited Paper)** [9166-22]
- 9166 0Q **A portable surface plasmon resonance biosensor capable of phase interrogation in a large dynamic range (Invited Paper)** [9166-24]
- 9166 0R **Optofluidic cellular immunofunctional analysis by localized surface plasmon resonance (Invited Paper)** [9166-25]

SESSION 4 BIOSENSING III

- 9166 0T **Nanoscale fiber tip probe for biomedical sensing (Invited Paper)** [9166-27]
- 9166 0Z **Probing the nano-bio interface with nanoplasmonic optical probes (Invited Paper)**
[9166-33]

POSTER SESSION

- 9166 11 **Effect of amine functionalized polyethylene on clay-silver dispersion for polyethylene nanocomposites** [9166-35]
- 9166 12 **Generation of reactive oxygen species from 5-aminolevulinic acid and Glutamate in cooperation with excited CdSe/ZnS QDs** [9166-36]
- 9166 13 **Enhancement of singlet oxygen production based on FRET between Coumarin tri-compound and CdSe/ZnS QDs** [9166-37]
- 9166 15 **Multivariate system of polarization tomography of biological crystals birefringence networks** [9166-39]
- 9166 16 **System of the phase tomography of optically anisotropic polycrystalline films of biological fluids** [9166-40]
- 9166 17 **Laser system of the autofluorescence polarimetry of cytological layers at an early stage of cancer detection** [9166-41]
- 9166 18 **Fluorescent biopsy of biological tissues in differentiation of benign and malignant tumors of prostate** [9166-42]
- 9166 19 **Statistical and fractal analysis of autofluorescent myocardium images in posthumous diagnostics of acute coronary insufficiency** [9166-43]
- 9166 1A **Polarization-correlation analysis of maps of optical anisotropy biological layers** [9166-44]
- 9166 1C **Optical pre-clinical diagnostics of the cervical tissues malignant changing** [9166-46]
- 9166 1D **Diagnostic value spectropolarimetry of blood plasma in patients with breast cancer**
[9166-47]
- 9166 1E **Absorption spectra of adenocarcinoma and squamous cell carcinoma cervical tissues**
[9166-48]
- 9166 1F **Dynamics of blood plasma by spectropolarimetry and biochemical techniques** [9166-49]

Authors

Numbers in the index correspond to the last two digits of the six-digit citation identifier (CID) article numbering system used in Proceedings of SPIE. The first four digits reflect the volume number. Base 36 numbering is employed for the last two digits and indicates the order of articles within the volume. Numbers start with 00, 01, 02, 03, 04, 05, 06, 07, 08, 09, 0A, 0B...0Z, followed by 10-1Z, 20-2Z, etc.

Abouzeid, Abraham H., 06
Ahmed, Tahsin, 0H
Ahn, Wonmi, 0L
Alonova, Marina, 1E
Amin, Hayder, 0N
Arnold, S., 0O
Bachinskiy, V. T., 19
Barnes, Eugenia, 05
Berdondini, Luca, 0N
Berry, Keith, 0L
Betancourt, Tania, 05
Blake, Phillip, 0L
Blanco-Canosa, Juan B., 0A
Blascakova, Ludmila, 04
Bohn, Paul, 0H
Boichuk, T. M., 19
Bradburne, Christopher E., 0A
Chang, Yun-Hsiang, 0Q
Chen, Chih-Hang, 0Q
Chen, How-Foo, 0Q
Cheung, Karen C., 0M
Chrostowski, Lukas, 0M
Chuang, Hsin-Yuan, 0Q
Dantham, V. R., 0O
Dawson, Philip E., 0A
De Angelis, Francesco, 0N
DeJarnette, Drew, 0L
Delehanty, James B., 0A
Dipalo, Michele, 0N
Donzella, Valentina, 0M
Dubolazov, A. V., 1A
Dunklin, Jeremy, 0L
Duong, Hong Dinh, 12, 13
Fedoruk, Olexander, 1C
Feizpour, Amin, 0Z
Flueckiger, Jonas, 0M
Forcherio, Gregory T., 0L
Foster, Erick, 0H
Garazdiuk, M., 19
Grist, Samantha M., 0M
Gritsyuk, M. V., 17, 18
Gruia, Ion, 1C
Gruia, Maria, 1F
Holler, S., 0O
Hong, W., 0T
Howard, Scott S., 0H
Ibarra-A, M. C., 11
Ilashchuka, Tetjana, 1F
Ivashko, Pavlo, 1D, 1E
Jancura, Daniel, 04
Jang, Gyoung G., 0L
Joniova, Jaroslava, 04
Karachevtsev, A. O., 16
Keng, D., 0O
Khan, Aamir A., 0H
Khanehzar, Ali, 0Z
Khater, Yashika, 07
Khumwan, Pakapreud, 0M
Kirk, James, 0M
Kolchenko, V., 0O
Koval, G. D., 17
Kruk, Tetjana, 1D
Kulik, Pavel, 0M
Kulkarni, Ashish, 07
Kurabayashi, Katsuo, 0R
La Rocca, Rosanna, 0N
Ledezma-P, A. S., 11
Lee, Jee Won, 12, 13
Liang, F., 0T
Lisunova, Milana, 0L
Loncar, M., 0T
Maccione, Alessandro, 0N
Malerba, Mario, 0N
Marchuk, Y. F., 1A
Martinez-C, J. G., 11
Medintz, Igor L., 0A
Messina, Gabriele C., 0N
Miller, Sarah, 05
Minzer, O. P., 18, 19
Miskovsky, Pavol, 04
Motrich, A. V., 19, 1A
Mulroe, Brigid, 0O
Nadova, Zuzana, 04
Novakovskaya, O. Yu., 17, 18, 1A
Oh, Bo-Ram, 0R
Olar, O. V., 16
Pashkovskaya, N. V., 1A
Paspaley-Grbavac, M., 0O
Patel, Niravkumar R., 06
Pavlov, S. V., 15, 16
Peresunko, Olexander, 1D, 1E
Prasuhn, Duane E., 0A
Prydij, Olexander, 1F
Prysyazhnyuk, V. S., 1A
Quan, Q., 0T
Rachman, Ilya M., 06
Ramírez-V, E., 11
Ramos-V, L. F., 11

Ratner, Daniel, 0M
Reinhard, Björn M., 0Z
Rhee, Jong Il, 12, 13
Rodriguez-F, O. S., 11
Romero-G, J., 11
Roper, D. Keith, 0L
Sánchez-Valdes, S., 11
Sarkar, Suproteem K., 07
Savich, V. O., 15, 16
Schaak, D., 0T
Schmidt, Shon, 0M
Sengupta, Shiladitya, 07
Senn, Sean, 06
Shalabaeva, Victoria, 0N
Sidor, M. I., 17, 18
Simi, Alessandro, 0N
Sobko, O. V., 15, 16
Stewart, Michael H., 0A
Sureau, Franck, 04
Susumu, Kimihiro, 0A
Sutton, Melissa, 05
Talebi Fard, Sahba, 0M
Thompson, Emily R., 0M
Torchilin, Vladimir P., 06
Trifoniuk, L. I., 18
Ushakova, Olga, 1D
Ushenko, A. G., 15, 16
Ushenko, Yu. A., 17, 18, 1A
Vanchuliak, O. Ya., 19
Vigil, Genevieve, 0H
Voloshynska, Katerina, 1F
Voloshynskyi, Dmytro, 1C
Wang, Qian, 0M
Weigum, Shannon E., 05
Wu, Linxi, 0Z
Wu, WenXuan, 0M
Xu, Fangda, 0Z
Yermolenko, Sergey, 1C
Yu, X., 0Z
Zabolotna, N. I., 15, 16
Zelinska, Natalia, 1E
Zimnyakov, Dmitry, 1C

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- 4 Biosensing III
Hooman Mohseni, Northwestern University (United States)
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Polarization-correlation analysis of maps of optical anisotropy biological layers

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ABSTRACT

A new information optical technique of diagnostics of the structure of polycrystalline films of bile is proposed. The model of Mueller-matrix description of mechanisms of optical anisotropy of such objects as optical activity, birefringence, as well as linear and circular dichroism is suggested. The ensemble of informationally topical azimuthally stable Mueller-matrix invariants is determined. Within the statistical analysis of such parameters distributions the objective criteria of differentiation of films of bile taken from healthy donors and diabetes of type 2 were determined. From the point of view of probative medicine the operational characteristics (sensitivity, specificity and accuracy) of the information-optical method of Mueller-matrix mapping of polycrystalline films of bile were found and its efficiency in diagnostics of diabetes extent of type 2 was demonstrated. Considered prospects of applying this method in the diagnosis of cirrhosis.

Keywords: polarimetry, optical anisotropy biological layers, polarization-correlation analysis.

1. Introduction

Laser polarimetry techniques [1-10] 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 – appears to be 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 the development of the method of “azimuthally stable” Mueller-matrix mapping of optical anisotropy of bile films in the task of diabetes extent of type 2.

2. Brief theoretical background

Laser polarimetry techniques [1-10] 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 – appears to be 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 the development of the method of “azimuthally stable” Mueller-matrix mapping of optical anisotropy of bile films in the task of diabetes extent of type 2

The description of mechanisms of optical anisotropy characteristic of bile films identified the following relationship Mueller-matrix invariants with phase parameters (δ , θ) и amplitude ($\Delta\tau$, C) anisotropy [11-21]:

$$\begin{cases} M_{44} \sim \cos \delta; \\ \frac{\Delta M_{23,32}}{\sum M_{22,33}} \sim \tan \theta. \end{cases} \quad (1)$$

Here δ - phase shift between linearly polarized orthogonal components of light beam amplitude, θ - rotation angle of polarization plane of the transformed light beam.

$$M_{14} = (1 - \Delta\tau) \sin \delta. \quad (2)$$

$$M_{41} = 4\sqrt{\Delta\tau} \cos \delta \frac{C}{1 + C^2}. \quad (3)$$

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

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

Thus, having measured the parameters distributions ($q \equiv \begin{cases} M_{14;41;44} (m \times n); \\ \Delta M_{ik} = \frac{\Delta M_{23,32}}{\sum M_{22,33}} (m \times n) \end{cases}$), one can obtain azimuthally

stable information about optical anisotropy of proteins of blood plasma film.

3. Investigation technique and processing of Mueller-matrix images

The measurements of coordinate distributions of Mueller-matrix elements were performed in the setup of the standard Stokes-polarimeter. The detailed description of the optical scheme (fig.1) and main parts of experimental setup was presented in the series of research works [11,12].

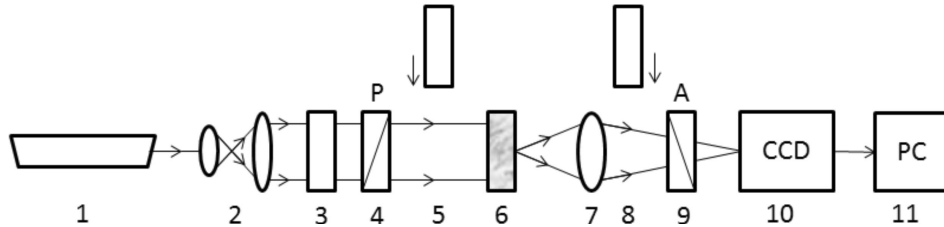


Fig. 1. Optical scheme of polarimeter, where 1 – He-Ne laser; 2 – collimator; 3 – stationary quarter-wave plate; 5, 8 – mechanically movable quarter-wave plates; 4, 9 – polarizer and analyzer respectively; 6 – object of investigation; 7 – polarization microobjective; 10 – CCD camera; 11 – personal computer.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

Illumination of samples 6 was performed by parallel ($\varnothing = 2 \times 10^3 \mu m$) low intensive ($W=5,0$ mW) beam of He-Cd laser ($\lambda = 0,414 \mu m$). Polarization light source consists of quarter-wave plates 3, 5 and polarizer 4. The investigated histological section 6 was sequentially probed by laser beam with the following polarization types: linear with azimuths 0° , 90° , $+45^\circ$ and right circulation (\otimes). The obtained images were projected on the plane of light sensitive plate ($m \times n = 800 \times 600$ pixels) of CCD-camera 10 by means of polarization microobjective 7 (focal distance $f = 30$ mm, magnification 4X, numerical aperture $NA = 0.1$). The analysis of the images of histological sections 6 was performed by means of polarizer 9 and quarter-wave plate 8. For the series of linearly (0° ; 45° ; 90°) and right- (\otimes) 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

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

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

Further the “informationally topical” parameters (10),(11) were calculated using the algorithm

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

4. Analysis and discussion of experimental data

Optically thin (attenuation factor $\tau < 0.1$) donors bile films dried at room temperature (group 1 – 25 patients) and those of breast cancer patients (group 2 – 25 patients) were used as objects of investigation.

Such distributions are illustrated by a series of coordinate dependences represented in Figures 2-5.

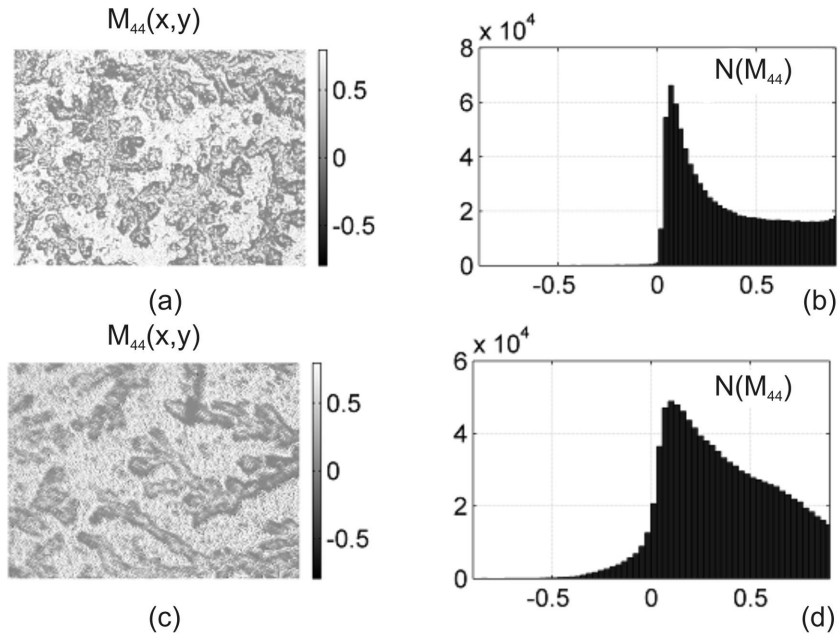


Fig. 2. Coordinate distributions of Mueller-matrix rotation invariant M_{44} , characterizing linear birefringence of films of donor's blood plasma ((a) – (b)) and of cancer patient ((c) - (d)).

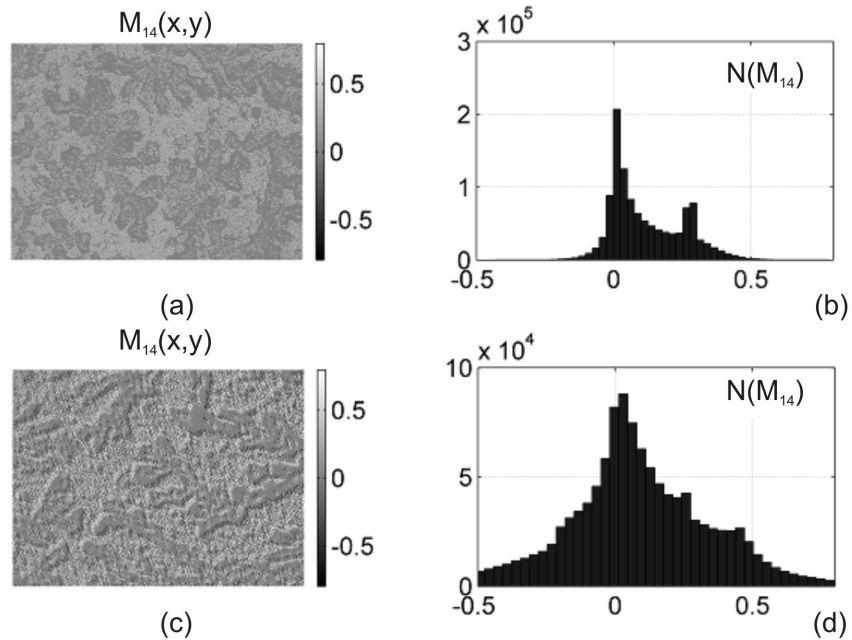


Fig. 3. Coordinate distributions of Mueller-matrix rotation invariant M_{14} , characterizing linear dichroism of films of donor's blood plasma ((a) – (b)) and of cancer patient ((c) - (d)).

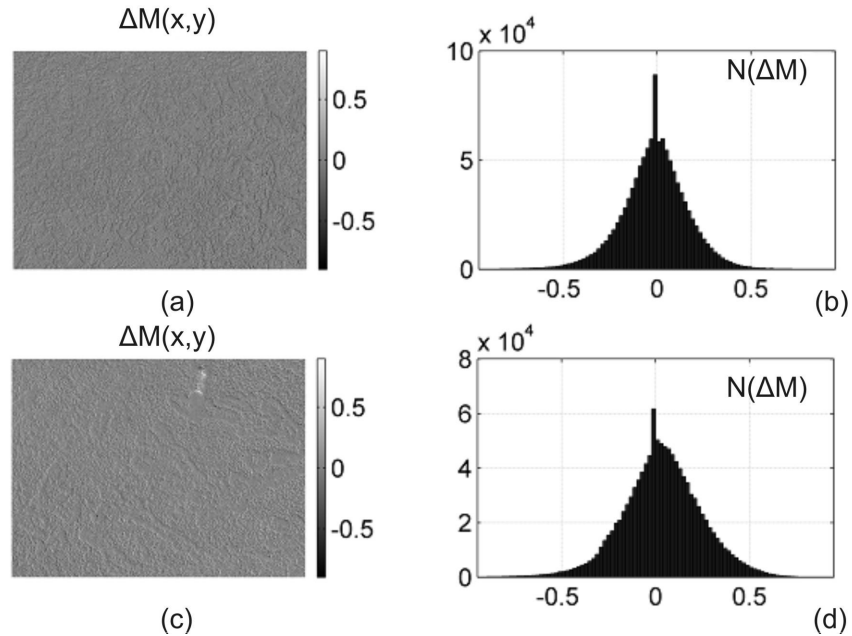


Fig. 4. Coordinate distributions of Mueller-matrix rotation invariant ΔM_{ik} , characterizing circular birefringence of films of donor's blood plasma ((a) – (b)) and of cancer patient ((c) - (d)).

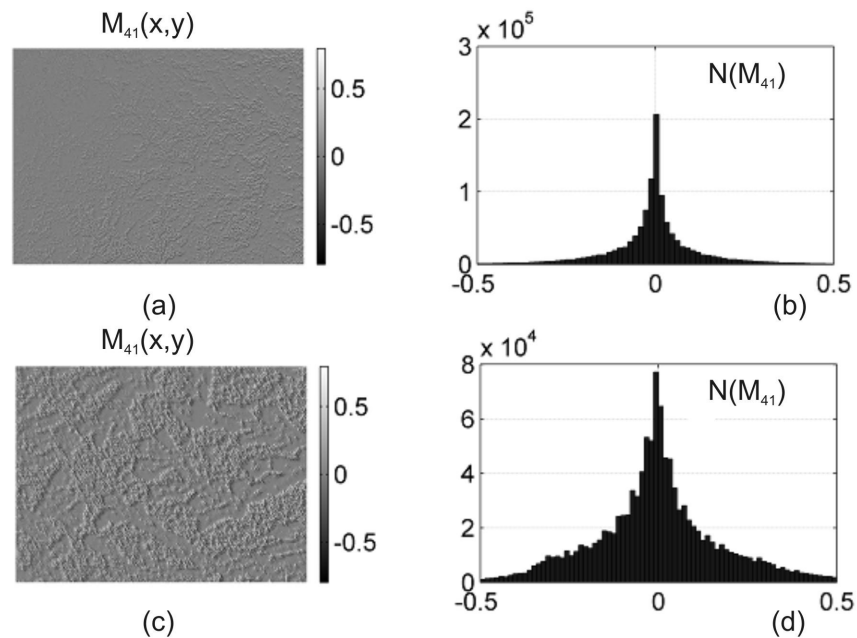


Fig. 5. Coordinate distributions of Mueller-matrix rotation invariant M_{41} , characterizing circular dichroism of films of donor's blood plasma ((a) – (b)) and of cancer patient ((c) - (d)).

The comparative analysis of the data obtained showed:

- *Linear birefringence* (Fig. 2). Distributions $M_{44}(m \times n)$ found for samples of both types are characterized by commensurable range of values change and rather identical coordinate structure. This fact can be related to that close in values to phase shifts δ determined by simultaneous contribution of the value of birefringence.
- *Linear dichroism* (Fig. 3). It is determined that protein network of donor's bile film has greater level of linear dichroism in comparison with similar sample of diabetic patient. The differences found between Mueller-matrix images $M_{14}(m \times n)$ can be related to the differences in concentration of basic proteins of bile.
- *Circular birefringence* (Fig. 4). It is defined that distributions ΔM_{ik} of the film of bile diabetic type II patients exceed the similar dependences for the samples from group 1.
- *Circular dichroism* (Fig. 5). Optically the anisotropic manifestations of tertiary structure of molecules are more vividly manifested for polycrystalline network of the film of bile of a healthy person. The range of changes of the value of matrix element M_{41} of the sample from group 1 are greater than in Mueller-matrix image of the sample from group 2.

The results of quantitative statistical analysis of a series of Mueller-matrix rotational invariants of two groups of bile films are illustrated by the data presented in Table 1.

Table 1. Statistical ($Z_{i=1;2;3;4}$) moments of distributions of Mueller-matrix invariants of films of bile of group 1 (normal) and group 2 (diabetes extent of type 2)

Parameters	M_{44}		M_{14}		ΔM_{ik}		M_{41}	
	Normal	Diabetes	Normal	Diabetes	Normal	Diabetes	Normal	Diabetes
Z_1	$0,34 \pm 0,0$ 61	$0,26 \pm 0,0$ 57	$0,23 \pm 0,0$ 38	$0,11 \pm 0,0$ 11	$0,14 \pm 0,0$ 24	$0,21 \pm 0,0$ 38	$0,12 \pm 0,0$ 18	$0,08 \pm 0,0$ 11
Z_2	$0,17 \pm 0,0$ 18	$0,21 \pm 0,0$ 24	$0,22 \pm 0,0$ 33	$0,15 \pm 0,0$ 19	$0,16 \pm 0,0$ 19	$0,12 \pm 0,0$ 21	$0,21 \pm 0,0$ 46	$0,12 \pm 0,0$ 18
Z_3	$0,23 \pm 0,0$ 43	$0,31 \pm 0,0$ 61	$0,78 \pm 0,1$ 4	$1,23 \pm 0,3$ 9	$0,97 \pm 0,1$ 2	$0,73 \pm 0,0$ 59	$1,17 \pm 0,1$ 5	$1,71 \pm 0,5$ 3
Z_4	$0,22 \pm 0,0$ 29	$0,27 \pm 0,0$ 38	$0,95 \pm 0,1$ 1	$1,34 \pm 0,4$ 7	$1,46 \pm 0,1$ 6	$0,81 \pm 0,0$ 65	$1,42 \pm 0,2$ 2	$2,15 \pm 0,7$ 3

The most sensitive parameters of differentiation of optically anisotropic networks of bile proteins of both groups are highlighted by grey colour in Table 1.

For possible clinical application of Mueller-matrix mapping within the investigated groups of samples the operational characteristics typical for probative medicine are: sensitivity ($Se = \frac{a}{a+b} \cdot 100\%$), specificity

($Sp = \frac{c}{c+d} \cdot 100\%$) and accuracy ($Ac = \frac{Se + Sp}{2}$), where a and b - the number of correct and wrong diagnoses within group 1; c and d - the same within group 2 – Table 2.

Table 2. Operational characteristics of Mueller-matrix mapping techniques

Parameters	M_{44}	M_{14}	ΔM_{ik}	M_{41}
$Se(Z_i), \%$	74	84	86	88
$Sp(Z_i), \%$	66	75	74	76
$Ac(Z_i), \%$	70	79	80	82

Thus, the statistical and correlation analyses of Mueller-matrix invariants characterizing polarization manifestations of linear dichroism, circular birefringence and circular dichroism appeared to be efficient in the task of differential diagnostics of diabetes extent of type 2.

5. Perspectives for other clinical applications

This method method was tested in the problem of diagnosing liver disease (cirrhosis) man. The following results – Table. 3.

Table 3. Operational characteristics of Mueller-matrix mapping techniques

Parameters	M_{44}	M_{14}	ΔM_{ik}	M_{41}
$Se(Z_i),\%$	86	80	92	78
$Sp(Z_i),\%$	78	74	84	74
$Ac(Z_i),\%$	82	77	88	76

CONCLUSIONS

1. The Mueller-matrix invariants characterizing polarization manifestations of partial mechanisms of optical anisotropy – linear birefringence, linear dichroism, circular birefringence and circular dichroism of polycrystalline film of blood plasma are determined.
2. Within the statistical and correlation approaches the interconnections between the set of moments of the 1st-4th orders and peculiarities of optically anisotropic networks of proteins of bile of healthy people and diabetes extent of type 2 patients are defined.
3. The efficiency of the technique of azimuthally invariant Mueller-matrix mapping of polycrystalline films of bile in the task of diabetes extent of type 2diagnostics is demonstrated.

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