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Letter

3D Mueller-matrix-based azimuthal invariant tomography of polycrystalline structure within benign and malignant soft-tissue tumours

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Abstract

We introduce a method of azimuthally invariant 3D Mueller-matrix (MM) layer-by-layer mapping of the phase and amplitude parameters of anisotropy of the partially depolarizing layers of benign (adenoma) and malignant (carcinoma) prostate tumours. The technique is based on the analysis of spatial variations of Mueller matrix invariant (MMI) of histological sections of benign (adenoma) and malignant (carcinoma) tissue samples. The phase dependence of magnitudes of the first-to-fourth order statistical moments is applied to characterize 3D spatial distributions of MMI of linear and circular birefringence and dichroism of prostate tumours. The high order statistical moments and phase sections of the optimal differentiation of the polycrystalline structure of tissue samples are revealed. The obtained results are compared with the results obtained by conventional methods utilizing polarized light, including 2D and 3D Mueller matrix imaging.

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(Some figures may appear in colour only in the online journal)

1. Introduction

Presently, the methods and means of polarisation-based diagnostics of biological tissues are extensively used in biomedical optics [1–4], including a number of subjects, such as: investigation of scattering matrices [5–9], polarization modeling of Monte Carlo [10–13], polarization spectroscopy [14–16], Mueller-matrix (MM) polarimetry [17–21], polar decomposition of MMs [22, 23], 2D MM mapping [24–27] within the framework of various model approximations [28–31] and other.

The variety of these polarization methods combines following common points:

• The use of the MM formalism [4, 6, 9, 15, 17, 32, 33].
• The resulting MMs are represented either in 1D (‘indicatrices’ [2, 5, 8, 9, 14, 19]) or in 2D (‘MM images’ [3, 18, 20, 21, 34, 35]) formats.
• The azimuthal dependence upon rotation of the sample around the axis of the 12th probe of the 16 elements of the MM [17–21, 24, 36, 37].
• Studies of MMs is carried out within the framework of two boundary approximations—optically thin (nondepolarizing [19–21]) and diffuse (depolarizing [3, 19, 26, 27, 34, 35]) objects.
• Almost complete lack of information about 3D distributions of matrix elements [32, 33].

Therefore, it is actually to develop 3D azimuthally invariant MM polarimetry of the most common type of objects—partially depolarizing biological tissues. The basis for this can be the joint use of probing and reference coherent beams. This will ensure the possibility of obtaining layered MM images based on the synthesis of methods:

• 2D mapping of Mueller-matrix invariants (MMIs) of biological layers—azimuthally independent distributions of matrix elements or their analytical combinations [24, 36, 37];
• Digital holographic restoration of distributions of complex amplitudes of the object field of such layers [32, 33].

Our article is aimed at the development and experimental validation of the diagnostic effectiveness of the method of azimuthally invariant MM tomography of the polycrystalline structure of histological sections of biopsy of benign and malignant tumors of the prostate.

2. Brief theory of the method

In [24, 35–39], it was shown that the following matrix elements and their combinations are azimuthally invariant.

Figure 1. Optical scheme of polarization interferometry of 3D distributions of MM elements. Explanations are in the text.

\[
\mathbf{F} = \begin{bmatrix}
F_{11} & F_{12} & F_{13} & F_{14} \\
F_{21} & F_{22} & F_{23} & F_{24} \\
F_{31} & F_{32} & F_{33} & F_{34} \\
F_{41} & F_{42} & F_{43} & F_{44}
\end{bmatrix} \Rightarrow \begin{bmatrix}
F_{11}(\Theta) = \text{const}; \\
F_{14}(\Theta) = \text{const}; \\
F_{41}(\Theta) = \text{const}; \\
F_{44}(\Theta) = \text{const};
\end{bmatrix}
\]

where \(\Theta\) is the angle of rotation of the sample.

In [40–46], the interrelations between the MMI (relation (1)) and averaged over the thickness (I) of the biological layer by 2D parameters of linear (LB) and circular (CB) birefringence.

\[
\begin{align*}
\Delta \mathbf{M} &= \mathbf{M}_{33;32} \sim \tan \text{CB} \\
\end{align*}
\]

and dichroism (LD, CD)

\[
\mathbf{F}_{41} = (1 - \text{LD}) \sin \text{LB}
\]

\[
\mathbf{F}_{41} = 4\sqrt{\text{LD} \cos \text{LB}} \frac{\text{CB}}{1 + \text{CB}^2}
\]
of light-sensitive plate—7600 × 6200 μm; sensitivity—0.05 lx; dynamic range—8 bit, SNR—9 bit. Reference laser beam by means of reflected mirror 4 is directed through the polarization filter 10–12 in the plane of polarization-inhomogeneous image of sample 8. As the result the interference pattern registered by digital camera 14 is formed. Formation of different polarization states of illuminating and reference beams is created by both polarization filters 5–7 and 10–12, each of them consists of two linear polarizers (B + W Kaesemann XS-Pro Polarizer MRC Nano) and quarterwave plate (Achromatic True Zero-Order Waveplate).

The technique of polarization-interference measurements of MM elements consists in the following sequence of operations:

- Formation of six polarization states ((0° − 0°); (90° − 90°); (45° − 45°); (135° − 135°); (0° − Ω); (Ω − Ω)) in both illuminating and reference laser beams. Here Ω denotes right and left circularly transmitted light correspondingly.
- Registration of each partial interference pattern after passing through analyzer-photodetector 13 with following sequence of orientations of transmitted laser beams: Ω = 0°; Ω = 90°.

Thus, by experimental measurement of the set of coordinate distributions \( q(x, y) \equiv F_{14,41,44}(x, y) \), it is possible to obtain azimuthally invariant information about the polarization manifestations of the phase and amplitude anisotropy of biological tissues.

\[
\Delta M(\phi_j) = \frac{(VS_{15}^6 - VS_{15}^{35}) - (VS_{15}^6 - VS_{15}^{95})}{(VS_{25}^6 - VS_{25}^{95}) + (VS_{35}^{65} - VS_{35}^{15})} (\phi_j) = \\
= \left[ \left( |A_1|^2 - |A_2|^2 \right) (45) - \left( |A_1|^2 - |A_2|^2 \right) (135) - 2 \left( Re |A_1 A_1^*|^{(0)} - Re |A_2 A_2^*|^{(90)} \right) \right] (\phi_j)
\]

\[
F_{14}(\phi_j) = \left( VS_{15}^6 - VS_{15}^{95} \right) (\phi_j) = \left( |A_1|^2 + |A_2|^2 \right) (0) - \left( |A_1|^2 + |A_2|^2 \right) (90) (\phi_j)
\]

\[
F_{41}(\phi_j) = \left( VS_{15}^6 + VS_{15}^{90} \right) (\phi_j) = 2 \left( Im |A_1 A_1^*|^{(0)} + Im |A_2 A_2^*|^{(90)} \right) (\phi_j)
\]

The optical scheme and the technique of experimental measurements of the MMI aggregate of biological layers (relations (6)–(9)) are described in detail and presented in [19–21, 24, 36, 37].

The basis for the determination of a series of a layered distributions of \( q(x, y, z) \) is the use of a reference laser wave, which is superimposed on the polarization-inhomogeneous image of the biological layer [31, 32].

The resulting interference pattern is recorded with a digital camera.

Further, apply Fourier transform to interference pattern and using the inverse Fourier transform obtained the distributions of complex amplitudes |\( A_1 \)|; |\( A_2 \)|exp(\( \delta_1 - \delta_2 \)) in different phase planes (\( \phi_j = (\delta_1 - \delta_2) = \frac{2\pi}{\lambda} \); \( 0 \leq z \leq l \)) of the object field with an arbitrary step \( \Delta \phi_j = 0.1\lambda \).

In each phase plane \( \phi_j(x, y) \), for a series of planar (with azimuths 0°, 90°, 45°) and the right of circularly (Ω) polarized irradiating beams, the distributions of Stokes vector \( VS(x, y, z, \phi_j) \) and MMI are calculated.

\[
\begin{align*}
VS_1(0°, 90°, 45°, \phi_j) & = \left( |A_1|^2 + |A_2|^2 \right) (66+90+45, \phi_j) \\
VS_2(0°, 90°, 45°, \phi_j) & = \left( |A_1|^2 - |A_2|^2 \right) (66+90+45, \phi_j) \\
VS_3(0°, 90°, 45°, \phi_j) & = 2 Re |A_1 A_1^*| (66+90+45, \phi_j) \\
VS_4(0°, 90°, 45°, \phi_j) & = 2 Im |A_1 A_1^*| (66+90+45, \phi_j)
\end{align*}
\]

(5)

\[
F_{44}(\phi_j) = \left( VS_4^6 \right) - \left( VS_4^9 \right) (\phi_j) = 2 \left( Im |A_1 A_1^*|^{(0)} - Im |A_2 A_2^*|^{(90)} \right) (\phi_j)
\]

(6)

3. Principles of 3D azimuthally-invariant MM differential diagnostics

To determine the diagnostic effectiveness of azimuthally-invariant 3D MM tomography, two representative groups of patients were formed with 28 samples of tumors in each. For each operably extracted tumor 16 histological sections were prepared with the geometric dimensions which are commensurable with the diameter of illuminating laser beam (~2000 × 2000 μm): (i) benign tumor (adenoma—16 samples)—group 1 (attenuation coefficient 0.79, personal experience 0.85, degree of depolarization 33% < A < 48%); (ii) malignant
Figure 2. Coordinate distributions of MMI $F_{44}$, characterizing the linear birefringence $LB$ of adenoma samples (fragment (1)) and carcinomas (fragment (2)) of the prostate.

4. Analysis and discussion of the experimental results

In figures 2–4 shows maps of the phase cross sections ($\phi_i$) of the MMI distributions ($m \times n = 100\, \text{pix} \times 100\, \mu m$; $1\, \text{pix} = 10\, \mu m$) 

- $F_{44}$ ($\phi_i = 0.45\, \text{rad}, m \times n$) (figure 1);
- $\Delta M$ ($\phi_i = 0.45\, \text{rad}, m \times n$) (figure 2);
- $F_{41}$ ($\phi_i = 0.75\, \text{rad}, m \times n$) (figure 3) and $F_{14}$ ($\phi_i = 0.75\, \text{rad}, m \times n$) (figure 4).

The MMI $F_{44}$; $\Delta M$ ($m \times n$) maps are obtained for the $\phi_i = 0.45\, \text{rad}$ phase section. Coordinate distributions of MMI $F_{41}$; $F_{14}$ ($m \times n$) are obtained for $\phi_i = 0.75\, \text{rad}$.

Let us analyze the results obtained from the physical point of view.

For the distributions of linear $LB$ (figure 1) and circular $CB$ (figure 2) birefringence of samples of both types due to different pathology of the prostate tissue, the following transformations of the polycrystalline structure take place.

A benign tumor contains a developed newly formed linear birefringent network, which is formed due to an increase in the concentration of optically active protein molecules [2–4, 19–21, 34, 35].

Oncological processes are accompanied by destruction of the fibrillar network. Optically, this is manifested in a decrease in the structural anisotropy—linear birefringence ($LB$ ↓) of the prostate tissue. Due to this, the mean and range of variation of the random variables of the MMI distribution $F_{44}$ ($\phi, m \times n$) $\sim$ $\cos LB$ (relation (2)) increases (figure 2, fragments (1), (2)). In parallel with this, the concentration of optically active protein molecules decreases due to necrosis. As a result, for the distribution $\Delta M$ ($\phi, m \times n$) $\sim$ $\tan CB$ (relation (2)), there is a reverse scenario—a decrease in the mean and range of variation of the random variables of this MMI (figure 2, fragments (1), (2)).

Within the framework of the statistical analysis of MMI distributions of the phase anisotropy of prostate tumors, one can
Figure 3. Coordinate distributions of MMI $\Delta M$, characterizing the circular birefringence $CB$ of adenoma samples (fragment (1)) and carcinomas (fragment (2)) of the prostate.

Figure 4. Coordinate distributions of MMI $F_{41}$, characterizing the linear dichroism $LD$ of adenoma samples (fragment (1)) and carcinomas (fragment (2)) of the prostate.

expect the following relationships between statistical moments [19, 24, 35, 38–43].

\[
\begin{align*}
Z_{1:2}^{\text{group}1}(F_{44}) &\prec Z_{1:2}^{\text{group}2}(F_{44}); \\
Z_{3:4}^{\text{group}1}(F_{41}) &\prec Z_{3:4}^{\text{group}2}(F_{44}); \\
Z_{1:2}^{\text{group}1}(\Delta M) &\prec Z_{1:2}^{\text{group}2}(\Delta M); \\
Z_{3:4}^{\text{group}1}(\Delta M) &\prec Z_{3:4}^{\text{group}2}(\Delta M).
\end{align*}
\]

(11)

The most pronounced such scenario (relations (11), (12)) is realized in the scattering region of insignificant multiplicity, which corresponds to small values ($0.3 \text{ rad} \prec \phi \prec 0.6 \text{ rad} \Rightarrow \phi^* = 0.45 \text{ rad}$) of the phase cross sections of the MMI $[F_{44}; \Delta M]$ ($\phi^*, m \times n$).

For the linear ($LD \sim \sin LB$ (relation (3))) dichroism of the partially depolarizing layers of adenomas and carcinomas (figures 4 and 5) of the prostate decreases

\[
\begin{align*}
Z_{1:2}^{\text{group}1}(F_{41}) &\succ Z_{1:2}^{\text{group}2}(F_{41}); \\
Z_{3:4}^{\text{group}1}(F_{41}) &\prec Z_{3:4}^{\text{group}2}(F_{41}); \\
Z_{1:2}^{\text{group}1}(F_{14}) &\succ Z_{1:2}^{\text{group}2}(F_{14}); \\
Z_{3:4}^{\text{group}1}(F_{14}) &\prec Z_{3:4}^{\text{group}2}(F_{14}).
\end{align*}
\]

(12)

The greatest difference between statistical moments $Z_{i=1,2,3,4}(F_{44};F_{41};F_{14})$ is achieved in the range of somewhat larger values of the ‘phase’ cross sections ($0.6 \text{ rad} \prec \phi \prec 0.9 \text{ rad} \Rightarrow \phi^* = 0.75 \text{ rad}$).

Table 1 presents the statistical criteria for differentiating benign and malignant prostate tumors using 3D azimuthally invariant MM tomography $[F_{44}; \Delta M; F_{41}; F_{14}] (\phi, m \times n)$. 

The circular ($CD \uparrow$, (equation (4))) dichroism of oncological changed prostate layers—increases
differentiation of benign and malignant prostate tumors with the comparative results of the diagnostic efficiency of the methods of polarization, 2D and 3D MM mapping.

5. Comparative characteristics of the diagnostic efficiency of the methods of polarization, 2D and 3D MM mapping

The comparative results of the diagnostic efficiency of differentiation of benign and malignant prostate tumors with different depolarizing ability by azimuthally invariant polarization [19, 38, 42–44, 46], 2D [20, 21, 24, 35, 38, 39, 41, 45] and 3D MM mapping. For this purpose, groups (16 samples each) of histological sections of adenoma biopsies and carcinomas with various geometric (l, µm), optical (τ) and depolarizing (Λ, %) characteristics were studied.

Table 2 shows the maximum values of balanced accuracy, which are achieved by the methods considered.

From the comparative analysis of the accuracy of the polarimetric methods for diagnosing the changes in the phase and amplitude anisotropy of the depolarizing layers of benign and malignant prostate tumors:

(a) Polarization mapping of the azimuth (α) and ellipticity (β) polarization of the microscopic images of biological preparations ensures satisfactory accuracy of differentiation of adenoma and prostate carcinoma (Ac(τ = 0.25) = 76% – 77%) to an insignificant level of depolarization (Λ = 28%); for large values Λ this method is ineffective (Ac < 70%).

(b) As the optical thickness (0.25 < τ < 0.62 ⇐ 28% < Λ < 43%) of the samples increases, the accuracy of the method of azimuthally-invariant 2D MM mapping

![Figure 5](image-url) Figure 5. Coordinate distributions of MMI \( F_{14} \), characterizing the circular dichroism \( CD \) of adenoma samples (fragment (1)) and carcinomas (fragment (2)) of the prostate.

| Parameters | Adenoma | Carcinoma | Accuracy, Ac, % |
|------------|---------|-----------|-----------------|
| MMI (\( \phi^* = 0.45 \text{ rad} \)) | \( F_{14} \) | \( \Delta M \) | \( F_{14} \) | \( \Delta M \) | \( F_{14} \) |
| \( Z_3 \) | 0.29 ± 0.017 | 0.12 ± 0.007 | 0.44 ± 0.029 | 0.07 ± 0.004 | 85 | 82 |
| \( Z_2 \) | 0.21 ± 0.012 | 0.15 ± 0.008 | 0.14 ± 0.006 | 0.11 ± 0.005 | 81 | 79 |
| \( Z_1 \) | 0.46 ± 0.029 | 0.63 ± 0.041 | 0.69 ± 0.037 | 0.92 ± 0.055 | 91 | 89 |
| \( Z_4 \) | 0.57 ± 0.033 | 0.88 ± 0.053 | 1.03 ± 0.059 | 1.39 ± 0.084 | 92 | 87 |
| MM (\( \phi^* = 0.75 \text{ rad} \)) | \( F_{14} \) | \( F_{14} \) | \( F_{14} \) | \( F_{14} \) | \( F_{14} \) | \( F_{14} \) |
| \( Z_3 \) | 0.210 ± 013 | 0.0750 ± 0042 | 0.15 ± 0.091 | 0.093 ± 0.005 | 81 | 73 |
| \( Z_2 \) | 0.14 ± 0.008 | 0.09 ± 0.005 | 0.095 ± 0.006 | 0.12 ± 0.071 | 79 | 77 |
| \( Z_1 \) | 0.77 ± 0.043 | 0.98 ± 0.052 | 1.18 ± 0.063 | 0.61 ± 0.037 | 92 | 91 |
| \( Z_4 \) | 0.96 ± 0.054 | 1.31 ± 0.079 | 1.65 ± 0.093 | 0.99 ± 0.056 | 94 | 93 |

The most sensitive to the differences between the polycrystalline structure of the adenoma samples and the prostate carcinoma parameters have been identified—the statistical moments of the third and fourth orders, which characterize the asymmetry and kurtosis of the distributions [\( F_{14}; \Delta M; F_{14}; \Delta M \) (\( \phi^*; m \times n \))].

For these parameters, according to the canons of evidence-based medicine [47], an excellent level of balanced accuracy of the 3D azimuthally invariant MM tomography method for partially depolarizing (\( \Lambda \leq 50\% \)) layers of biological tissues in differential diagnosis of benign and malignant prostate tumors was achieved — Ac(\( Z_{3,4}; F_{14,41,14}; \Delta M \)) ~ 91% – 94%.

The result stimulated an additional study—the determination of the comparative efficacy of traditional [1–4, 12–15, 19–21, 24, 34, 35, 38–43] and the methods of polarimetric diagnostics proposed in this work, depending on the depolarizing ability of biological tissue samples.
Table 2. Comparative diagnostic effectiveness of azimuthal invariant polarimetry methods.

| \( \tau \) | \( 0.25 \) | \( 0.45 \) | \( 0.62 \) |
|----------|--------|--------|--------|
| \( l, \mu m \) | 20     | 40     | 50     |
| \( \Lambda, \% \) | 28     | 35     | 43     |
| \( Ac, \% \{\alpha,\beta\} \) | 2D 3D | 2D 3D | 2D 3D |
| \( Ac(Z_e) \) | 73     | 78     | 91     |
| \( Ac(Z_e) \) | 76     | 79     | 93     |
| \( Ac(Z_e) \) | 76     | 84     | 95     |
| \( Ac(Z_e) \) | 77     | 83     | 96     |

| \( \tau \) | \( 0.91 \) | \( 1.37 \) | \( 1.93 \) |
|----------|--------|--------|--------|
| \( l, \mu m \) | 60     | 80     | 100    |
| \( \Lambda, \% \) | 52     | 68     | 89     |
| \( Ac, \% \{\alpha,\beta\} \) | 2D 3D | 2D 3D | 2D 3D |
| \( Ac(Z_e) \) | 59     | 63     | 83     |
| \( Ac(Z_e) \) | 58     | 65     | 85     |
| \( Ac(Z_e) \) | 61     | 68     | 91     |
| \( Ac(Z_e) \) | 63     | 69     | 94     |

| decreasing from good \( (Ac (\tau = 0.25; \Lambda = 28 \%) = 83\% - 84\%) \) to satisfactory \( (Ac (\tau = 0.62; \Lambda = 43\%) = 75\%) \).

(c) Azimuthal invariant 3D MM tomography proved to be the most diagnostically effective \( (70\% < Ac < 96\%) \) in a wide range of optical thickness \( (0.25 < \tau < 1.37 \Rightarrow 28\% < \Lambda < 68\%) \) histological sections of biopsy of benign and malignant tumors of the prostate.

6. Conclusion

A method of azimuthally-invariant 3D MM tomography of phase and amplitude anisotropy of partially depolarizing layers of the prostate benign and malignant tumors is proposed and justified.

Layered ‘phase’ dependences of the statistical moments of the 1st-4th order, which characterize the M3M \{\( F_{44}; \Delta M; F_{43}; F_{41} \)\} \( \{\phi, m \times n\} \) of the polycrystalline component of the histological sections of prostate tumors, were investigated.

Optimal conditions for differentiation of polycrystalline structures of benign and malignant tumors—the range of phase sections \( \{\phi^{\ast}(F_{44}; \Delta M) = 0.45 \text{rad}; \phi^{\ast}(F_{41}; F_{44}) = 0.75 \text{rad} \} \) and the most sensitive parameters \( \Delta Z_{i=3,4}^{\ast}(F_{44}; \Delta M; F_{43}; F_{41}) \equiv \Delta Z_{i=3,4}^{\ast}(\phi^{\ast}) \rightarrow \max \) (statistical moments of the 3rd and 4th orders) are revealed.

Statistical analysis of the distributions of the parameters of phase \( (L_B, C_B) \) and amplitude \( (L_D, C_D) \) anisotropy \( \{\phi^{\ast}, m \times n\} \) provided an excellent good balanced accuracy \( (Ac(Z_{3,4}, [F_{44}; \Delta M; F_{43}; F_{41}]) \sim 91\% - 94\%) \) of differential diagnosis of prostate tumors.

Comparative studies of the diagnostic efficiency of azimuthally-invariant methods of polarization, 2D and 3D MM mapping of prostate tumor samples with different optical thickness \( (\tau) \) and depolarizing ability \( (\Lambda) \) were carried out. On this basis, the levels of balanced accuracy \( (Ac) \) and limits \( (0.25 < \tau < 1.37 \Rightarrow 28\% < \Lambda < 68\%) \) of using the methods are determined.

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The presenting authors do not have any financial conflicts of interest regarding the content of this manuscript.

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