

Winter College on Optics: Advanced Optical Techniques for Bio-imaging

POLARIZATION MICROSCOPY: BIOMEDICAL IMAGING AND DIAGNOSTICS

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Date & time:

(Lecture) February 17, 2017 (Friday), 15.30 (Experiment) February 23, 2017 (Thursday), 14.00 Room: Leonardo Building - Budinich Lecture Hall

LIST OF LECTURES

• Introduction.

- Lecture 1. Basic concepts. Polarization. Stokes vector. Mueller matrix. Basics of laser polarimetry.
- Lecture 2. Basics of model description of structure and optical anisotropy of biological tissues.
- Lecture 3. Methods and resources of analysis and processing of biological tissues polarization-inhomogeneous images.
- Lecture 4. Principles and methods of polarization and Mueller-matrix mapping.











INTRODUCTION

- Optical methods of diagnostics of biological objects and visualization of their structures occupy a leading position thanks to their high information content, multi-functional capabilities (photometric, spectral, and polarization correlation).
- It should be stated that new scientific direction optics of biological tissues and fluids was finally formed and rapidly developing. The main areas of basic research are the results of theoretical and experimental studies of photon transport in biological tissues and fluids.
- A separate direction in optics of biological tissues formed polarimetric investigations. Analysis of polarization characteristics of the scattered radiation allows to obtain qualitatively new results on morphological and physiological state of biological tissues.
- A new step in the development of methods of optical diagnostics of biological tissues was successful unification of polarimetric and fluorescent techniques.

Light as transverse electromagnetic wave

The electric and magnetic fields of an electromagnetic wave are perpendicular to each other and transverse to the direction of propagation. An electromagnetic wave is propagating along z-axis. Its electric field is aligned to the x-axis and magnetic field along the y-axis.





 δ – phase (initial)

Polarization of electromagnetic wave

Polarization is a important property of electromagnetic waves. In communications, completely polarized waves are used. In radio astronomy unpolarized components exist. The techniques to analyze polarization known as *polarimetry*.

The complete polarization types of electromagnetic waves are:
(i) Linear Polarization.
(ii) Circular Polarization.
(iii) Elliptical Polarization.

Electromagnetic waves from of radio astronomical sources may posses: (i) Random polarization (also known as un-polarized waves). (ii) Partial polarization (completely polarized + un-polarized)

Polarization of electromagnetic wave. Graphical representation. **Polarization ellipse**



equation of an ellipse

An ellipse can be characterized by:

- size of minor axis 1
- 2. size of major axis
- 3. orientation (tilt angle, azimuth)
- 4. Axial ratio (ellipticity)
- 5. sense (CW, CCW)

Axial ratio - is a ratio of length of minor to the length of major axis.

$$\varepsilon = \pm \arctan(\frac{OB}{OA})$$

- ellipticity (angle)

Polarization of electromagnetic wave. Types of polarization. Linear polarization

Any form of complete polarization resulting from a coherent source can be analyzed using *polarization ellipse* !!!



Polarization of electromagnetic wave. Types of polarization. Circular polarization



Polarization of electromagnetic wave. Types of polarization. Elliptical polarization.

If the magnitudes of **E**x and **E**y are not equal, and there exists a phase difference between the two, the tip of the electric field vector describes an ellipse and wave is said to be *elliptically polarized*.



Stokes parameters

1852: Sir George Gabriel Stokes took a <u>very different approach</u> and discovered that polarization can be described in terms of <u>observables</u> using an <u>experimental definition</u>.

The polarization ellipse is only valid at a given instant of time (function of time)!!!

$$\left(\frac{\mathrm{E}_{\mathrm{x}}}{\mathrm{E}_{0\mathrm{x}}}\right)^{2} + \left(\frac{\mathrm{E}_{\mathrm{y}}}{\mathrm{E}_{0\mathrm{y}}}\right)^{2} - 2\frac{\mathrm{E}_{\mathrm{x}}}{\mathrm{E}_{0\mathrm{x}}}\frac{\mathrm{E}_{\mathrm{y}}}{\mathrm{E}_{0\mathrm{y}}}\cos\delta = \sin^{2}\delta$$

To get the Stokes parameters, do a time average (integral over time) and a little bit of algebra...

$$\left(E_{0x}^{2} + E_{0y}^{2}\right)^{2} - \left(E_{0x}^{2} - E_{0y}^{2}\right)^{2} - \left(2E_{0x}E_{0y}\cos\delta\right)^{2} = \left(2E_{0x}E_{0y}\sin\delta\right)^{2}$$

The 4 Stokes parameters are:

$$S_0 = I = E_{0x}^2 + E_{0y}^2$$
$$S_1 = Q = E_{0x}^2 - E_{0y}^2$$
$$S_2 = U = 2E_{0x}E_{0y}\cos\delta$$
$$S_3 = V = 2E_{0x}E_{0y}\sin\delta$$

Stokes parameters described in geometrical terms. Stokes vector



$$\begin{pmatrix} I \\ Q \\ U \\ V \end{pmatrix} = \begin{pmatrix} 1 \\ \cos 2\varepsilon \cos 2\tau \\ \cos 2\varepsilon \sin 2\tau \\ \sin 2\varepsilon \end{pmatrix}$$

The Stokes parameters can be arranged in a Stokes vector:

$$\begin{pmatrix} I \\ Q \\ U \\ U \\ V \end{pmatrix} = \begin{pmatrix} E_{0x}^{2} + E_{0y}^{2} \\ E_{0x}^{2} - E_{0y}^{2} \\ 2E_{0x}E_{0y}\cos\delta \\ 2E_{0x}E_{0y}\sin\delta \end{pmatrix} = \begin{pmatrix} \text{intensity} \\ I(0^{\circ}) - I(90^{\circ}) \\ I(45^{\circ}) - I(135^{\circ}) \\ I(RCP) - I(LCP) \end{pmatrix}$$

- Linear polarization
- Circular polarization
- Fully polarized light

 $Q \neq 0, U \neq 0, V = 0$ $Q = 0, U = 0, V \neq 0$ $I^{2} = Q^{2} + U^{2} + V^{2}$

Mueller matrices

If light is represented by Stokes vectors, optical components are then described with Mueller matrices:

[output light] = [Muller matrix] [input light]





Need to be measured

$$I_{\min}\begin{pmatrix} r_1, \dots, r_m \\ \dots, \dots, r_m \end{pmatrix}; I_{\max}\begin{pmatrix} r_1, \dots, r_m \\ \dots, \dots, r_m \end{pmatrix}$$

$$\Theta\begin{pmatrix}r_1,\ldots,r_m\\\ldots,\ldots\\r_n,\ldots,r_m\end{pmatrix} \left(I\begin{pmatrix}r_1,\ldots,r_m\\\ldots\\\ldots\\r_n,\ldots,r_m\end{pmatrix} = \min \right)$$

Polarization maps (calculation)

$$\alpha \begin{pmatrix} r_1, \dots, r_m \\ \dots, \dots, r_m \end{pmatrix} = \Theta(I(r_i) \equiv \min) - \frac{\pi}{2};$$

$$\beta \begin{pmatrix} r_1, \dots, r_m \\ \dots, \dots, r_m \end{pmatrix} = \operatorname{arctg} \frac{I(r_i)_{\min}}{I(r_i)_{\max}}.$$

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Optical anisotropy

Optical anisotropy - difference in the optical properties of a medium as a function of the direction of propagation of optical radiation (light) in the medium and of the state of polarization of the radiation.

Amplitude anisotropy (dichroism)

 crystals may similarly show absorption which depends upon polarization.

1. Linear dichroism 2. Circular dichroism

Phase anisotropy (birefringence)

- asymmetry in crystal structure causes two different refractive indices;
- opposite polarizations follow different paths through crystal.
 - Linear birefringence
 Circular birefringence (optical activity)



Mueller-matrix approach for description of biological layers with amplitude and phase anisotropies

Soft tissue structure





Biological tissues reveal selfsimilar (fractal) structure as a result of growth processes **Transmission electron micrograph of human** skin (dermis), showing collagen fibers sectioned both longitudinally and transversely. Magnification 4900x.

Mueller-matrix approach for description of biological layers with amplitude and phase anisotropies

Optically anisotropic biological layer						
Phase a	nisotropy	Amplitude anisotropy				
Optical activity	Linear	Circular dichroism	Linear dichroism			
	birefringence					
Parameters						
Polarization plane	Phase shift between	Circular dichroism	Linear dichroism			
rotation angle - θ	the orthogonal	index - Δg	index - Δau			
	components of					
	amplitude - δ					
Partial Mueller	Partial Mueller	Partial Mueller	Partial Mueller			
matrix - $\{\Omega\}$	matrix - $\{D\}$	matrix - $\{\Phi\}$	matrix - $\{\Psi\}$			
Mueller matrix of generalized anisotropy						
$\{M\} = \{\Omega\} \times \{D\} \times \{\Phi\} \times \{\Psi\}$						
Azimuthally independent Mueller-matrix elements and invariants						
$M_{ik}(\Theta) = const ; \Delta M_{ik}(\Theta) = const$						
Algorithms of Mueller-matrix reconstruction of parameters of optical anisotropy						
$0 \dots (M^* \wedge M^*)$	S (14* A14*)					

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 $\theta = w(M_{ik}^*, \Delta M_{ik}^*) \qquad \delta = u(M_{ik}^*, \Delta M_{ik}^*) \qquad \Delta g = h(M_{ik}^*, \Delta M_{ik}^*) \qquad \Delta \tau = v(M_{ik}^*, \Delta M_{ik}^*)$

Algorithm of Mueller-matrix modeling of biological layer anisotropy

Mueller-matrix operators of mechanisms of phase and amplitude anisotropy

Circular birefringence

Linear birefringence

$$\{\Omega\} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \omega_{22} & \omega_{23} & 0 \\ 0 & \omega_{32} & \omega_{33} & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \qquad \omega_{ik} = \begin{cases} \omega_{22} = \omega_{33} = \cos 2\theta, \\ \omega_{23} = -\omega_{32} = \sin 2\theta. \end{cases}$$

Circular dichroism

$$\{\Phi\} = \begin{vmatrix} 1 & 0 & 0 & \phi_{14} \\ 0 & \phi_{22} & 0 & 0 \\ 0 & 0 & \phi_{33} & 0 \\ \phi_{41} & 0 & 0 & 1 \end{vmatrix} \qquad \phi_{ik} = \begin{cases} \phi_{22} = \phi_{33} = \frac{1 - \Delta g^2}{1 + \Delta g^2}, \\ \phi_{14} = \phi_{41} = \pm \frac{2\Delta g}{1 + \Delta g^2}. \end{cases}$$

Linear dichroism

$$\{D\} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & d_{22} & d_{23} & d_{24} \\ 0 & d_{32} & d_{33} & d_{34} \\ 0 & d_{42} & d_{43} & d_{44} \end{bmatrix}$$

$$d_{ik} = \begin{cases} d_{22} = \cos^2 2\rho + \sin^2 2\rho \cos \delta, \\ d_{23} = d_{32} = \cos 2\rho \sin 2\rho (1 - \cos \delta), \\ d_{33} = \sin^2 2\rho + \cos^2 2\rho \cos \delta, \\ d_{42} = -d_{24} = \sin 2\rho \sin \delta, \\ d_{42} = -d_{24} = \sin 2\rho \sin \delta, \\ d_{44} = \cos \delta. \end{cases}$$

$$\{\Psi\} = \begin{bmatrix} 1 & \psi_{12} & \psi_{13} & 0 \\ \psi_{21} & \psi_{22} & \psi_{23} & 0 \\ \psi_{31} & \psi_{32} & \psi_{33} & 0 \\ 0 & 0 & 0 & \psi_{44} \end{bmatrix}$$

$$\varphi_{ik} = \frac{1}{1 + \Delta\tau} \times \begin{cases} \psi_{12} = \psi_{21} = (1 - \Delta\tau) \cos 2\gamma; \\ \psi_{13} = \psi_{31} = (1 - \Delta\tau) \sin 2\gamma; \\ \psi_{22} = (1 + \Delta\tau) \cos^2 2\gamma + 2\sqrt{\Delta\tau} \sin^2 2\gamma; \\ \psi_{23} = \psi_{32} = (1 - \sqrt{\Delta\tau})^2 \cos 2\gamma \sin 2\gamma; \\ \psi_{33} = (1 - \sqrt{\Delta\tau})^2 \cos 2\gamma \sin 2\gamma; \\ \psi_{33} = (1 - \Delta\tau) \sin^2 2\gamma + 2\sqrt{\Delta\tau} \cos^2 2\gamma; \\ \psi_{44} = 2\sqrt{\Delta\tau}. \end{cases}$$

Generalized Mueller matrix of biological tissue optical anisotropy

$$M = \{\Omega\} \times \{D\} \times \{\Phi\} \times \{\Psi\} \qquad \{M\}$$

$$\{M\} = M_{11}^{-1} \times \begin{vmatrix} 1 & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{vmatrix}$$

Mueller-matrix approach for description of biological layers with amplitude and phase anisotropies



Mueller-matrix images of skeletal muscle

Information content of Mueller-matrix elements

$$M_{i=1;k=1;2;3;4}$$

- Mechanisms of optically anisotropic absorption



- Phase modulation (δ, θ) of laser radiation on the background of optically anisotropic absorption ($\Delta g, \Delta \tau$)



- Complex information about superposition of mechanisms of linear birefringence and dichroism

Mueller-matrix invariants

$$\begin{pmatrix} M_{11}(\Theta) = const; M_{14}(\Theta) = const, \\ M_{41}(\Theta) = const; M_{44}(\Theta) = const, \end{pmatrix}$$

$$\frac{M_{23} - M_{32}}{M_{22} + M_{33}} = \Delta M = const$$



$$M_{14} \propto \Delta \tau$$

$$M_{41} \propto \Delta g$$

$$M_{44} = \cos \delta$$

$$\Delta M = tg 2\theta.$$

Samples structure (histological sections) 1. Tissue with ordered and disordered structure





Myocardium tissue in coaxial and crossed polarizer-analyzer



Brain tissue in coaxial and crossed polarizer-analyzer

Samples structure (histological sections) 2. Parenchymatous tissue with cluster (disordered) structure



Benign tumor (adenoma) of prostate gland tissue in coaxial and crossed polarizer-analyzer

Samples structure (histological sections) 3. Tissues with benign and malignant formations



Precancer (dysplasia) of *cervix uteri* tissue



Malignant formation (adenocarcinopma) of *cervix uteri* tissue

Samples structure (dried smears of biological fluids)



Donor blood plasma in coaxial and crossed polarizer-analyzer



Synovial fluid of joint with rheumatoid arthritis in coaxial and crossed polarizer-analyzer

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LECTURE 3. METHODS AND RESOURCES OF ANALYSIS AND PROCESSING OF BIOLOGICAL TISSUES POLARIZATION-INHOMOGENEOUS IMAGES.

Objective criteria

All the data and parameters (q) presented in previous lectures need to be quantitatively analyzed!!! It was proposed to use:

1. Statistic analysis

$$\boxed{Z_1 = \frac{1}{N} \sum_{i=1}^{N} (q)_i} - \text{Mean value}$$

A moment is a quantitative measure of the shape of a set of points.



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2. Correlation analysis

Correlation plays a central role in the study of time series. In general, correlation gives a quantitative estimate of the degree of similarity between two *functions.* The correlation of functions g and f both with N samples is defined

as:

$$r_{k} = \frac{1}{N} \sum_{i=0}^{N-k-1} g_{i} f_{k+i}$$

$$k = 0, 1, 2, \dots, N-1$$

Auto-correlation – correlation of a signal with itself.





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2. Correlation analysis

Any azimuthally asymmetric distribution can be evaluated by correlation analysis in two perpendicular directions. Based on this, we used the following methodology of autocorrelation processing of the distribution of values q:



$$\begin{cases} K(W, X, y) \rightarrow \begin{pmatrix} K_{y=1}(\Delta x); \Delta x = 1, \dots, m \\ \dots, \dots, K_{y=n}(\Delta x); \Delta x = 1, \dots, m \end{pmatrix} \rightarrow \overline{K}(\Delta x) = \frac{1}{n} \sum_{i=1}^{n} K_i(\Delta x); \\ K(W, x, Y) \rightarrow \begin{pmatrix} K_{x=1}(\Delta y); \Delta y = 1, \dots, n \\ \dots, \dots, K_{y=m}(\Delta y); \Delta y = 1, \dots, n \end{pmatrix} \rightarrow \overline{K}(\Delta y) = \frac{1}{m} \sum_{i=1}^{n} K_i(\Delta y).$$

Half-width of autocorrelation dependency is an important diagnostical characteristic!!!

$$\xi = \frac{P_{max}}{P_{min}} - Asymmetry coefficient$$

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3. Fractal (self-similarity) analysis

Fractal anlysis is based on the calculation of logarithmic dependencies of power spectra of values $q lg L(q) - lg(d^{-1})$. Further mentioned dependecies are approximated by least squares method in a curves $\Phi(\eta)$.

Due to the form of curves $\Phi(\eta)$ one can classify:

1. Distributions q are fractal when there is one stable inclination angle $\eta = const$ within 2-3 decades of sizes changes.

2. Distributions *q* are multifractal when there is several stable inclination angles exist.

3. Distributions *q* are random when there is no stable inclination angles.



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Linear polarization with azimuths 0⁰, 45⁰, 90⁰

Right circular polarization

azimuths 0⁰, 45⁰, 90⁰, 135⁰, right and left circular

Experimental setup and measuring technique

Let the probing beam will be linearly polarized with azimuth $\alpha_0 = 0^0$.

First step: set transmission plane of analyzer 9 at an angle of $\Theta = 0^{\circ}$ and measure $I_0(m \times n)$ set transmission plane of analyzer 9 at an angle of $\Theta = 90^{\circ}$ and measure $I_{90}(m \times n)$



Second step: set transmission plane of analyzer 9 at an angle of $\Theta = 45^{\circ}$ and measure $I_{45}(m \times n)$ set transmission plane of analyzer 9 at an angle of $\Theta = 135^{\circ}$ and measure $I_{135}(m \times n)$



$$S_3 = (I_{45} - I_{135}) / S_1$$





Experimental setup and measuring technique

Third step: insert quarter-wave plate 8 set transmission plane of analyzer 9 at an angle of $\Theta = 45^{\circ}$ and measure $I_{\otimes}(m \times n)$ set transmission plane of analyzer 9 at an angle of $\Theta = 135^{\circ}$ and measure $I_{\oplus}(m \times n)$



Similarly one can calculate other Stokes vectors: $S_{i=1;2;3;4}^{45,90,\otimes}(m \times n)$

Algorithm for Mueller matrix calculation

Stokes vector parameters

 $S_{i=1}^{0;45;90;\otimes} = I_0^{0;45;90;\otimes} + I_{90}^{0;45;90;\otimes};$ $S_{i=2}^{0;45;90;\otimes} = I_0^{0;45;90;\otimes} - I_{90}^{0;45;90;\otimes};$ $S_{i=3}^{0;45;90;\otimes} = I_{45}^{0;45;90;\otimes} - I_{135}^{0;45;90;\otimes}; \qquad M_{14} = S_1^{\otimes} - f_{11};$ $S_{i-4}^{0;45;90;\otimes} = I_{\infty}^{0;45;90;\otimes} + I_{\infty}^{0;45;90;\otimes}, \qquad M_{21} = 0.5(S_2^0 + S_2^{90});$

Mueller-matrix elements

 $M_{11} = 0.5(S_1^0 + S_1^{90});$ $M_{12} = 0.5(S_1^0 - S_1^{90});$ $M_{13} = S_1^{45} - f_{11};$ $M_{22} = 0.5(S_2^0 - S_2^{90});$ $M_{22} = S_2^{45} - f_{21};$ $M_{24} = S_2^{\otimes} - f_{21};$

$$M_{31} = 0.5(S_3^0 + S_3^{90});$$

$$M_{32} = 0.5(S_3^0 - S_3^{90});$$

$$M_{32} = 0.5(S_3^0 - S_3^{90});$$

$$M_{33} = S_3^{45} - f_{31};$$

$$M_{34} = S_3^{\otimes} - f_{31};$$

$$M_{41} = 0.5(S_4^0 + S_4^{90});$$

$$M_{42} = 0.5(S_4^0 - S_4^{90});$$

$$M_{42} = 0.5(S_4^0 - S_4^{90});$$

$$M_{43} = S_4^{45} - f_{41};$$

$$M_{44} = S_4^{\otimes} - f_{41}.$$

n parameters

Mueller-matrix invariants and optical anisotropy parameters



Circular birefringence



Mueller-matrix invariants and optical anisotropy parameters





Mueller-matrix invariants and optical anisotropy parameters

Parameters	$M_{_{44}}$	ΔM	$M_{_{14}}$	$M_{_{41}}$
Z_1	0.46	0.12	0.73	0.16
Z_2	0.29	0.13	0.19	0.11
Z_{3}	0.48	0.23	0.57	1.14
Z_4	0.47	0.61	0.41	0.93
D	0.23	0.29	0.26	0.22