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distribution formed by Fourier hologram [12126-70] 1212610 Registration of three-dimensional holograms based on microsystems "Core CaF2 –Shell AgBr" [12126-71] 12126 1P Extraordinary transverse spin: hidden vorticity of the energy flow and momentum distributions in propagating light fields [12126-72] 12126 1Q Energy and momentum of the surface plasmon-polariton supported by a thin metal film [12126-73] 12126 1R Optical control of colour deviation due to ink showing through on the banknote reverse on multitoned watermarks [12126-74] 12126 IS Electrical properties of photosensitive ZnO/Si heterostructure depending on temperature [12126-75] 12126 IT Evidence for the need to update the definition of the BRDF: spectral considerations [12126-76] 12126 10 UV sensitive heterojunction ZnCoO/n-GaP prepared by spray pyrolysis [12126-77] 12126 IV Influence of chromium sublayer on silicon P-I-N photodiodes responsivity [12126-78] 12126 1W Dynamic interferometry method for measuring wavelength [12126-79]

Fourier energy analysis of Kikuchi patterns for investigation of defect system of diamond

Stabilizing effect of random phase diffuser against wavefront distortions to the intensity

- 12126 1X Measurement of parameters of optically transparent films [12126-80]
- 12126 1Y Optical transfer matrix: matrix correlation as frequency domain analysis of polarization imaging system (Invited Paper) [12126-82]
- 12126 17 Algorithm for diagnosing pancreatic endocrine dysfunction based on biochemical and laser polarimetric parameters [12126-83]
- 12126 20 The effect of photonic correction on the optical and photoelectric characteristics of the In₄Se₃, In₄Te₃ and GaP epitaxial structures [12126-84]
- 12126 21 Forensic medical assessment of cerebral infarction, hemorrhagic hemorrhages of traumatic genesis and determination of the duration of their formation methods of spectral-selective laser-induced direct polarization-phase tomography [12126-86]
- Polarization mapping of laser-induced monospectral fields of optically anisotropic fluorophores in forensic diagnostics of the age of the formation of damage to human organs [12126-87]
- 12126 23 Mueller-matrix microscopy of laser-induced monochromatic fluorescent fields of preparations of human internal organs and histological diagnostics of the time of age of damage formation [12126-89]

12126 1M

12126 IN

crystals [12126-67]

Mueller-matrix microscopy of laser-induced monochromatic fluorescent fields of preparations of human internal organs and histological diagnostics of the time of age of damage formation

Litvinenko¹ A.Yu., Kvasnyuk¹ D., Vanchulyak¹ A.Ya., Stashkevich² M., Motrich³ A.V.,

Mikhailova³ A.Yu., Gorskiy³ M.P., Slyotov³ M.M. ¹Bukovinian State Medical University, Chernivtsi, Ukraine ²The Institute of Traumatology and Orthopedics by NAMS of Ukraine, Kyiv, Ukraine

³ Chernivtsi National University, Chernivtsi, Ukraine

m.gorskii@chnu.edu.ua

ABSTRACT

The article contains the results of experimental testing of methods of azimuthal-invariant Mueller-matrix microscopy (Mueller-matrix invariants - MMI) of optically anisotropic fluorophores of samples of histological sections of the brain, liver and kidney, as well as myocardium and lung tissue; temporal detection of variations in the magnitude of the statistical moments of the 1st - 4th orders, characterizing the distributions of the MMI value of linear birefringence and optical activity of samples of histological sections of the brain, liver and kidney, as well as myocardium and lung tissue with different age of damage; determination of the diagnostic efficiency (time interval and accuracy) of establishing the age of damage to human internal organs by digital histological methods of MMI mapping of optical anisotropy of fluorophores in histological sections of the brain, liver and kidney, as well as myocardium and lung tissue.

Keywords: polarization, Mueller matrix, linear birefringence, statistical moments of the 1st - 4th orders, histological sections, biological tissues, damages

1. INTRODUCTION

Methods and means of Mueller-matrix polarimetry [1-5] of biological tissues and fluids of human organs are described in detail in many works of groups of domestic (Dubolazov A.V., Ushenko Yu.) [6-15] and foreign (N. Gosh, A. Vitkin, Tuchin V. V. and others) scientists [16-19].

However, at present, these digital methods of polarizing Mueller-matrix microscopy are practically absent in histological studies of determining the age of damage to human internal organs. In addition, the analysis of microscopic images is performed semi-qualitatively by observing the structure of the image by an expert, followed by his subjective conclusion.

The aim of the study is to develop a set of objective forensic criteria for establishing the age of damage to human internal organs according to the data of the azimuthal-invariant Mueller matrix mapping of the polycrystalline structure of molecular domains-fluorophores of prototypes.

2. MATERIALS AND METHODS

The following groups were formed (control for those who died from coronary artery disease and experienced with different age of damage) of prototypes of histological sections of internal organs (brain, liver, kidney, as well as the myocardium and lung tissue) of a person [20-26].

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Internal organ				Grou	ıps					
Brain,	Control		Exp	erienced	l with di	fferent a	ge of da	mage, h	ours	
Liver, Kidney, Myocardium,	Deceased from CAD(21)	1	6	12	18	24	48	72	96	120
Lung tissue		21	21	21	21	21	21	21	21	21

The study design consisted of the fact that within each of the groups of samples:

- obtained MMI maps of linear and circular birefringence of molecular fluorophores;
- the statistical moments of the 1st 4th orders were calculated, characterizing the distributions of the value of the set of MMI;

• was determined within the control and the set of research groups, the average value and the error of the magnitude of each of the statistical moments of the 1st - 4th orders;

• the age of the damage and its accuracy were calculated algorithmically.

3. DIFFERENTIAL HISTOLOGICAL DIAGNOSTICS OF THE AGE OF FORMATION OF INJURIES OF HUMAN INTERNAL ORGANS BY MAPPING THE MULLER-MATRIX INVARIANT OF LINEAR BIREFRINGENCE

Coordinate ((1) - (3)) and statistical ((4) - (6)) structure of MMI of linear birefringence of histological sections of the brain with research ((1), (4)) and two control ((2), (3), (5), (6)) groups is shown in Fig. 1.

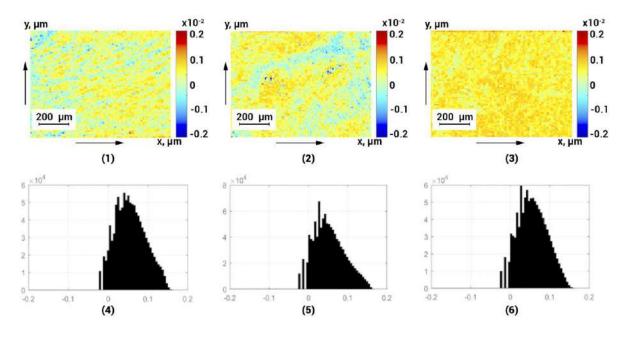


Fig. 1. Maps ((1), (2), (3)) and histograms ((4), (5), (6)) of the distributions of the MMI value of linear birefringence (x4) of histological sections of the brain of the deceased from the control group ((1), (4)), research groups with different duration of damage (6 hours - (2), (5)) and (18 hours - (3), (6)).

Mueller matrix microscopy of histological sections of the brain revealed:

• coordinate heterogeneity of all MMI maps, - fragments ((1) - (3));

• the presence of a significant scatter of MMI values in the corresponding histograms of distributions for different age of damage, - fragments ((4) - (6));

• a decrease in the level of crystallization of the brain substance with an increase in the age of damage - the average value and dispersion of the distributions of the MMI value of linear birefringence, calculated for the prescription of damage of 6 hours, decrease. (Fragment (5)) and 18 hours (Fragment (6)).

Quantitatively, the temporal transformation of the magnitude of the statistical moments of the 1st - 4th orders characterizing the distributions of the MMI of linear birefringence (4x) of brain samples is given in Table 1..

Analysis of the temporal dynamics of changes in the magnitude of the statistical moments, which are presented in Table 1, revealed diagnostic efficiency at a certain age of damage to internal organs of the following parameters:

• statistical moment of the 2nd order - the interval of linear and statistically significant ($p \prec 0.05$) change 6 hours with a dynamic range of 0.11;

• skewness and kurtosis - the duration of the linear interval up to 18 hours with a dynamic range of 0.84 and 0.72, respectively;

The greater diagnostic sensitivity of the Mueller-matrix microscopy technique was ensured by the use of large-scale (40x) polarization mapping of the degree of crystallization of histological sections of the brain of the control and a set of research samples of samples, - Fig. 2, table 2.

T, hours	2	4	6	12	18		
$SM_1 \times 10^{-1}$	0,42±0,027	0,39±0,023	0,37±0,016	0,35±0,015	0,33±0,014		
р		$p \succ 0.05$					
$SM_2 \text{ x10}^{-1}$	0,94±0,041	$0,83 \pm 0,038$	0,79±0,036	$0,74 \pm 0,034$	$0,71 \pm 0,035$		
р	$p\prec$	0,05		$p \succ 0,05$			
SM_3	0,39±0,019	$0,53 \pm 0,028$	0,67±0,031	$0,95 \pm 0,042$	$1,23 \pm 0,058$		
р			<i>p</i> ≺ 0,05				
SM_4	0,16±0,08	0,27±0,013	0,38±0,016	$0,62 \pm 0,032$	$0,88 \pm 0,041$		
р	$p \prec 0.05$						
T, hours	24	48	72	96	120		
$\frac{T, \text{ hours}}{SM_1 \text{ x}10^{-1}}$	24 0.36±0,019	48 0,34±0,018	72 0,32±0,017	96 0,33±0,018	120 0,32±0,017		
$SM_1 ext{ x10}^{-1}$			$0,32 \pm 0,017$				
$\frac{SM_1 \times 10^{-1}}{p}$	0.36±0,019	0,34±0,018	$0,32 \pm 0,017$ $p \succ 0,05$	0,33±0,018	0,32±0,017		
$\frac{SM_{1} \times 10^{-1}}{p}$ $\frac{SM_{2} \times 10^{-1}}{s}$	0.36±0,019	0,34±0,018	$0,32 \pm 0,017$ $p \succ 0,05$ $0,63 \pm 0,032$	0,33±0,018	0,32±0,017		
	0.36 ± 0.019 0.68 ± 0.035	$0,34 \pm 0,018$ $0,65 \pm 0,033$	$0,32 \pm 0,017$ $p \succ 0,05$ $0,63 \pm 0,032$ $p \succ 0,05$	$0,33 \pm 0,018$ $0,64 \pm 0,032$	$0,32 \pm 0,017$ $0,63 \pm 0,032$		
$\frac{SM_{1} \times 10^{-1}}{p}$ $\frac{SM_{2} \times 10^{-1}}{p}$ $\frac{SM_{3}}{s}$	0.36 ± 0.019 0.68 ± 0.035	$0,34 \pm 0,018$ $0,65 \pm 0,033$	$0,32 \pm 0,017$ $p \succ 0,05$ $0,63 \pm 0,032$ $p \succ 0,05$ $1,46 \pm 0,072$	$0,33 \pm 0,018$ $0,64 \pm 0,032$	$0,32 \pm 0,017$ $0,63 \pm 0,032$		

Table 1 Time dynamics of changes in statistical moments of the 1st - 4th orders, characterizing the distribution of MMI LB

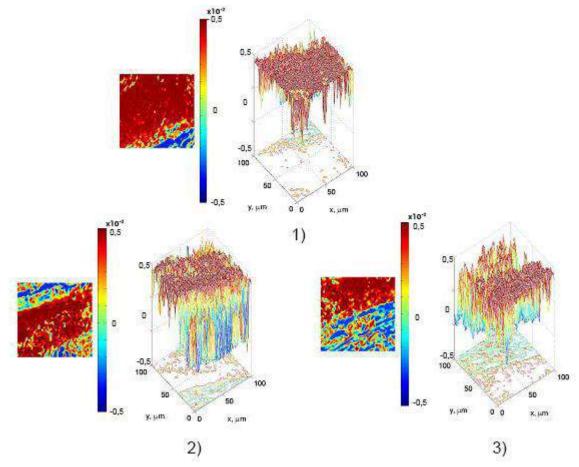


Fig. 2. Maps ((1), (2), (3)) and coordinate distributions ((4), (5), (6)) of the MMI value of linear birefringence (x40) of histological brain sections from the control group ((1), (4)), research groups with different age of damage (6 hours - (2), (5)) and (18 hours - (3), (6)).

Table 2 Time dynamics of changes in the statistical moments of the 1st - 4th orders characterizing the distributions of the MMI value of linear birefringence (40x) of histological brain sections

T, hours	2	4	6	12	18	
$SM_1 \mathrm{x10^{-1}}$	$0,34 \pm 0,014$	$0,25 \pm 0,011$	$0,23 \pm 0,012$	$0,22 \pm 0,012$	0,23±0,013	
р	$p\prec$	0,05		$p \succ 0,05$		
$SM_2 \text{ x10}^{-1}$	1,39±0,061	$1,08 \pm 0,048$	0,89±0,036	$0,84 \pm 0,034$	0,81±0,035	
р		<i>p</i> ≺ 0,05		$p \succ$	0,05	
SM ₃	0,29±0,013	0,41±0,018	$0,55 \pm 0,023$	$0,79 \pm 0,034$	$1,03 \pm 0,048$	
р			<i>p</i> ≺ 0,05			
SM_4	$0,13 \pm 0,06$	0,24±0,011	0,36±0,016	0,61±0,027	$0,84 \pm 0,038$	
р		$p \prec 0,05$				
T, hours	24	48	72	96	120	
$SM_1 \times 10^{-1}$	$0.22 \pm 0,013$	0,24±0,012	0,22±0,011	$0,23 \pm 0,012$	0,22±0,011	

р			<i>p</i> ≻ 0,05				
$SM_2 \text{ x10}^{-1}$	$0,83 \pm 0,045$	$0,85 \pm 0,043$	$0,83 \pm 0,042$	$0,84 \pm 0,042$	0,81±0,042		
р		$p \succ 0.05$					
SM ₃	1,24±0,11	$1,28 \pm 0,14$	$1,33 \pm 0,17$	1,39±0,15	1,44±0,12		
р	<i>p</i> ≺ 0,05		$p \succ$	0,05			
SM_4	$1,07 \pm 0,094$	1,11±0,12	1,15±0,13	1,19±0,15	1,14±0,11		
р	<i>p</i> ≺ 0,05	$p \succ 0,05$					

The dynamic ranges, as well as the diagnostic sensitivity to the age of brain damage, of statistical moments of the 1st-4th orders with the following time intervals of linear and statistically significant changes in eigenvalues have been established:

- 1st order statistical moment (average) 6 hours and 0,09;
- 2nd order statistical moment (dispersion) 12 hours and 0,05;
- 3rd order statistical moment (skewness) 24 hours and 0,74;
- 4th order statistical moment (kurtosis) 24 hours and 0,71.

From the analysis of the data obtained, an increase (up to 4) in the number of diagnostic-sensitive statistical parameters (average, dispersion, skewness and kurtosis of histograms of the distributions of MMI of the degree of crystallization of brain tissue) and the duration of the range of linear change in their magnitude from the age of damage were established...

4. DIFFERENTIAL HISTOLOGICAL DIAGNOSIS OF THE AGE OF THE FORMATION OF INJURIES OF HUMAN INTERNAL ORGANS BY MAPPING THE MUELLER-MATRIX INVARIANT OF CIRCULAR BIREFRINGENCE

Two-dimensional maps of the Mueller-matrix invariant of circular birefringence of histological sections of the brain ((1) - (3)) and histograms ((4) - (6)) of the distribution of its magnitude are shown in Fig. 3.

Comparison of the digital histology data of the degree of crystallization of the brain substance (Fig. 2) also revealed the topographic structure (Fig. 3, fragments (4) - (6)) of the distributions of the MMI CB value and coordinate heterogeneity (Fig. 3, fragments (1) - (3)).

It was also found that with an increase in the age of damage, the average and dispersion of the MMI CB decrease (Fig. 3, fragments (5) and (6)), and the skewness and kurtosis of such distributions, on the contrary, increase - Table 3.

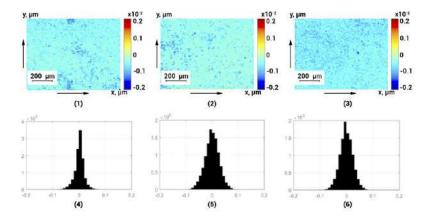


Fig. 3. Maps ((1), (2), (3)) and histograms ((4), (5), (6)) of the distributions of the MMI value of circular birefringence (x4) of histological sections of the brain of the deceased from the control group ((1), (4)), research groups with different duration of damage (6 hours - (2), (5)) and (18 hours - (3), (6)).

T, hours	2	4	6	12	18
SM_{1} x10 ⁻¹	0,19±0,009	0,17±0,008	0,16±0,008	$0,15 \pm 0,007$	0,13±0,006
р			<i>p</i> ≻ 0,05		
$SM_{2} \text{ x10}^{-1}$	$0,17 \pm 0,007$	$0,105 \pm 0,004$	$0,09 \pm 0,005$	$0,08 \pm 0,004$	$0,09 \pm 0,005$
р	$p \prec$	0,05		<i>p</i> ≻ 0,05	
SM ₃	$0,59 \pm 0,029$	$0,71 \pm 0,032$	0,83±0,039	$1,09 \pm 0,042$	$1,33 \pm 0,058$
р			<i>p</i> ≺ 0,05		
SM_4	$0,71 \pm 0,028$	0,97±0,043	$1,22 \pm 0,056$	$1,73 \pm 0,082$	1,98±0,091
р			<i>p</i> ≺ 0,05		
T, hours	24	48	72	96	120
$SM_1 \mathrm{x10^{-1}}$	$0.13 \pm 0,009$	0,14±0,008	$0,12 \pm 0,007$	$0,11 \pm 0,008$	$0,12 \pm 0,007$
р			<i>p</i> ≻ 0,05		
$SM_2 \text{ x10}^{-1}$	$0,08 \pm 0,005$	$0,065 \pm 0,003$	$0,063 \pm 0,003$	$0,064 \pm 0,003$	$0,063 \pm 0,003$
р			<i>p</i> ≻ 0,05		
SM ₃	$1,31 \pm 0,062$	$1,35 \pm 0,066$	1,46±0,072	1,39±0,065	$1,38 \pm 0,063$
р		-	<i>p</i> ≻ 0,05	•	
SM_4	1,92±0,098	2,06±0,097	2,11±0,11	2,13±0,12	2,14±0,12
р			<i>p</i> ≻ 0,05		

Table 3 Time dynamics of changes in the statistical moments of the 1st - 4th orders characterizing the distributions of the MMI value of circular birefringence (4x) of histological sections of the brain

An increase in the number of diagnostic parameters of this method by Mueller-matrix microscopy in comparison with the method of detecting the degree of crystallization of the brain substance is shown. Along with the intervals of linear change in the statistical moments of higher orders (18 hours, dynamic range 0.74 and 1.26), a linear change in the dispersion characterizing the spread of the MMI CB value was revealed over a time interval of up to 4 hours with a dynamic range of 0.055.

The results of the azimuthal-invariant Mueller matrix mapping of large-scale (40x) maps of the MMI CB of histological brain sections are presented in a series of fragments in Fig. 4.

Comparative analysis of the obtained data (Fig. 4) with small-scale maps of MMI CB (Fig. 3) revealed an increase in the range of variation in the magnitude of fluctuations in the values of optical activity of molecular complexes of brain samples, - Table 4.

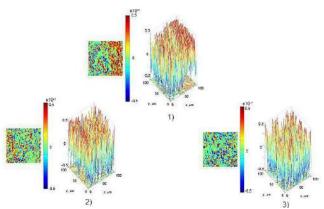


Fig. 4. Maps ((1), (2), (3)) and coordinate distributions ((4), (5), (6)) of the magnitude of MMI circular birefringence (x40) histological sections of the brain of the deceased from the control group ((1), (4)), research groups with different age of damage (6 hours - (2), (5)) and (18 hours - (3), (6)).

T, hours	2	4	6	12	18		
$SM_1 x 10^{-1}$	$0,16 \pm 0,07$	$0,11 \pm 0,004$	$0,09 \pm 0,005$	$0,08 \pm 0,007$	$0,085 \pm 0,006$		
р	$p \succ$	0,05					
$SM_2 \text{ x10}^{-1}$	$0,14 \pm 0,007$	$0,095 \pm 0,004$	$0,069 \pm 0,003$	$0,068 \pm 0,004$	$0,069 \pm 0,005$		
р		<i>p</i> ≺ 0,05		$p \succ$	0,05		
SM ₃	$0,65 \pm 0,029$	0,79±0,032	$0,93 \pm 0,043$	$1,29 \pm 0,054$	1,57±0,065		
р		$p \prec 0.05$					
SM_4	$0,87 \pm 0,038$	$1,17 \pm 0,053$	$1,49 \pm 0,065$	$2,07 \pm 0,098$	2,68±0,14		
р			<i>p</i> ≺ 0,05				
T, hours	24	48	72	96	120		
$SM_1 x 10^{-1}$	$0.068 \pm 0,003$	$0,049 \pm 0,002$	$0,037 \pm 0,001$	$0,034 \pm 0,002$	$0,029 \pm 0,001$		
р			<i>p</i> ≻ 0,05				
$SM_2 x 10^{-1}$	$0,068 \pm 0,005$	$0,065 \pm 0,003$	$0,063 \pm 0,002$	$0,064 \pm 0,002$	$0,063 \pm 0,002$		
р			<i>p</i> ≻ 0,05				
SM ₃	$1,85 \pm 0,17$	$1,89 \pm 0,18$	$1,96 \pm 0,17$	$1,99 \pm 0,95$	1,93±0,16		
р	<i>p</i> ≺ 0,05		$p \succ$	0,05	<u> </u>		
SM ₄	$3,02 \pm 0,26$	3,11±0,29	3,13±0,33	3,19±0,32	3,14±0,31		
р	<i>p</i> ≺ 0,05		$p \succ 0.05$				

Table 4 Time dynamics of changes in the statistical moments of the 1st - 4th orders characterizing the distribution of MMI circular birefringence (40x) of brain sections

The growth (all statistical moments of the 1st - 4th orders) of the number of diagnostic-sensitive parameters (average, dispersion, skewness and kurtosis of histograms of distributions of MMI CB of brain tissue) and the duration of the range of statistically significant ($p \prec 0.05$) linear changes in their magnitude from the age of damage was established:

• 1st order statistical moment (average) – 6 hours and 0,05;

• 2nd order statistical moment (dispersion) – 12 hours and 0,075;

- 3rd order statistical moment (skewness) 24 hours and 1,2;
- 4th order statistical moment (kurtosis) 24 hours and 2,15.

5. TIME INTERVALS AND ACCURACY OF DIGITAL HISTOLOGICAL DETERMINATION OF THE AGE OF DAMAGE BY THE METHODS OF AZIMUTHAL-INVARIANT MUELLER-MATRIX MAPPING

Table 5 Time intervals and accuracy of the method of polarization mapping of MMI maps of linear birefringence

Brain						
Statistical moments	Interva	al, hours	Accura	acy, min.		
Magnification	4x	40x	4x	40x		
Average,	_	4	_	70		
Dispersion,	6	6	65	60		
Skewness,	18	24	50	40		
Kurtosis,	18	24	50	40		
Myocardium						
Statistical moments	Interva	al, hours	Accura	acy, min.		
Magnification	4x	40x	4x	40x		
Average,	_	4	_	70		
Dispersion,	6	6	65	60		
Skewness,	18	24	50	40		
Kurtosis,	18	24	50	40		
	Live	er				
Statistical moments	Interva	al, hours	Accura	acy, min.		
Magnification	4x	40x	4x	40x		
Average,	_	4	_	70		
Dispersion,	6	6	65	60		
Skewness,	18	24	55	45		
Kurtosis,	18	24	55	45		
	Lung ti	issue				
Statistical moments	Interva	al, hours	Accura	acy, min.		
Magnification	4x	40x	4x	40x		
Average,	_	4	_	70		
Dispersion,	6	6	65	60		
Skewness,	18	24	55	45		
Kurtosis,	18	24	55	45		
	Kidn	ey				
Statistical moments	Interva	al, hours	Accura	acy, min.		
Magnification	4x	40x	4x	40x		
Average,	_	4	_	70		
Dispersion,	6	6	65	60		
Skewness,	18	24	50	40		
Kurtosis,	18	24	50	40		

Table 6 Time intervals and accuracy of the method of polarization mapping of MMI maps of circular birefringence

Brain							
Statistical moments Interval, hours Accuracy, min.							
Magnification	4x	40x	4x	40x			
Average,	_	4	_	65			
Dispersion,	6	6	60	55			

Skewness,	18	24	45	35
Kurtosis,	18	24	45	35
	Myocar	dium		
Statistical moments	Interv	al, hours	Accura	acy, min.
Magnification	4x	40x	4x	40x
Average,	_	4	_	65
Dispersion,	6	6	60	55
Skewness,	18	24	45	35
Kurtosis,	18	24	45	35
	Live			
Statistical moments	Interv	al, hours	Accura	acy, min.
Magnification	4x	40x	4x	40x
Average,	_	4	_	65
Dispersion,	6	6	60	55
Skewness,	18	24	50	40
Kurtosis,	18	24	50	40
	Lung ti	issue		
Statistical moments	Interv	al, hours	Accura	acy, min.
Magnification	4x	40x	4x	40x
Average,	_	4	_	65
Dispersion,	6	6	60	55
Skewness,	18	24	50	40
Kurtosis,	18	24	50	40
	Kidn			
Statistical moments	Interv	al, hours	Accura	acy, min.
Magnification	4x	40x	4x	40x
Average,	_	4	_	65
Dispersion,	6	6	60	55
Skewness,	18	24	45	35
Kurtosis,	18	24	45	35

CONCLUSIONS

1. By using a new digital histological technique based on azimuthal-invariant Muller-matrix mapping of optically anisotropic molecular fluorophores of samples of human internal organs with different periods of damage (brain, liver, kidney, as well as myocardium and lung tissue), changes in the morphological and biochemical structure have been studied for the first time on interval from 1 hour up to 120 hours.

2. For the first time, the main interrelationships between temporal changes in the statistical structure of maps of Mueller-matrix invariants of linear (degree of crystallization) and circular (optical activity) birefringence of molecular fluorophores in histological sections of human internal organs and variations in the magnitude of statistical moments of the 1st - 4th orders that characterize them have been revealed - with an increase in the age of damage, the value of the average and dispersion decreases, skewness and kurtosis, on the contrary, increase.

3. Diagnostic-sensitive parameters have been established - the time ranges of linear changes in the variations in the magnitude of the statistical indicators of the Mueller-matrix digital histology methods and the accuracy of determining the duration of damage:

3.1. MMI maps of crystallization degree (4x):

- dispersion 6 h.;
- skewness 18 h.;
- kurtosis 18 h.;

- accuracy 60 min. 70 min.
- 3.2. MMI maps of crystallization degree (40x):
 - average 4 h.;
 - dispersion 6 h.;
 - skewness 24 h.;
 - kurtosis 24 h..;
 - $\operatorname{accuracy} 55 \operatorname{min.} 60 \operatorname{min.}$

3.3. MMI optical activity maps (4x):

- dispersion 6 h.;
- skewness 18 h.;
- kurtosis 18 h.;
- accuracy 70 min. 80 min.

3.4. MMI optical activity maps (40x):

- average 4 h.;
- dispersion 6 h.;
- skewness– 24 h.;
- kurtosis– 24 h.;
- accuracy 65 min. 75 min.

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