ESTIMATION OF THE TIME SINCE DEATH BY USING THE POLARIZATION MULLER-MATRIX MICROSCOPY OF BIOLOGICAL TISSUE IN CASES OF ALCOHOL AND CARBON MONOXIDE POISONING

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Abstract:

Introduction: This paper aims to investigate and substantiate the information capabilities of the objective method of azimuthal-invariant polarization Müller-matrix microscopy of histological sections of biological tissues (BT) in the diagnosis of time since death (TSD) in alcohol and monoxide poisoning.

Objectives: The purpose of the study was to evaluate the diagnostic capabilities of the azimuthal-invariant Muller-matrix polarimetry technique of the polycrystalline constituent of BT and human body fluids in determining the TSD in cases of alcohol and carbon monoxide poisoning.

Results: A comprehensive experimental study of the diagnostic efficiency of the azimuthal-invariant Muller-matrix polarimetric microscopy of the polycrystalline component of histological sections of the brain, myocardium, adrenal glands, liver, and polycrystalline blood films from dead persons for the diagnosis of TSD in cases of alcohol and carbon monoxide poisoning. A sensitivity range of 24 hours was determined using the method of the azimuthal-invariant Muller-matrix polarimetric microscopy of the polycrystalline component of histological sections of the myocardium for the diagnosis of TSD in cases of alcohol and carbon monoxide poisoning.

Conclusion: The results showed the effectiveness of the studied methodology and the prospect of further research in this direction.

Introduction

Determining the TSD of a person continues to be one of the most important issues to be resolved during the forensic examination of a dead body. In the early post-mortem period, a wide variety of methods (subjective and objective) exist for solving this issue (Gonnade & Chavan, 2018; Shaaban El-Raouf et al., 2017; Tomé, 2017). However, there is currently no single method of assessment that can ensure the accuracy and reproducibility of the result. At the same time, attention is drawn to the lack of information on specific morphological changes in BT in various pathological conditions, such as, poisoning with alcohol and carbon monoxide (Hayden, 2019; Kim et al, 2018; Rose et al, 2017). Therefore, it is now necessary to develop and implement new methods for determining the time since death.

A new step was the application and development of laser polarimetric methods of microscopic examination of optically inhomogeneous biological structures using statistical analysis of digital polarization maps. The study of Ushenko (2015) confirmed that these methods provide almost complete information about the polycrystalline structure of BT and fluids of the human body and allow to examine the dynamics of changes in different pathological conditions for the diagnosis of the post-mortem interval.

Purpose

The purpose of the study was to evaluate the diagnostic capabilities of the azimuthal-invariant Muller-matrix polarimetric microscopy of the polycrystalline constituent of BT and human body fluids in determining the TSD in cases of alcohol and carbon monoxide poisoning.

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Materials and Methods

The object of the study was histological sections of the brain, liver, adrenal glands, myocardium and polycrystalline blood films, selected from 56 corpses of both sexes, aged 18 to 71 years, with a pre-known time of onset of death, ranging from 1 to 40 hours. All samples were divided into two experimental groups by cause of death: (i) due to ethanol poisoning (ii) due to carbon monoxide poisoning. BT samples from the persons deceased due to coronary heart disease were used as control group with a sample size of 17. The basic principle of our modeling process was based on the use of microscopic images of biological layers of human tissue and fluid, which were well-established Müller-matrix polarimetric analytical approach for the diagnosis of morphological and molecular work of BT. (Ushenko et al, 2016). According to this approach, the biological object is considered as a two-component ("isotropic-anisotropic") structural matrix.

The basis of developing objective digital criteria for forensic diagnostics of TSD in cases of alcohol and carbon monoxide poisoning is based on the azimuthal-invariant Muller-matrix polarimetry of the polycrystalline constituent of BT and fluids of the human body. The research methodology is illustrated by the following scheme (Figure 1).

Figure 1: Structural-logical scheme of the method of azimuthal-invariant mapping of the degree of crystallization of biological layers		
1	Source of optical sounding	Gas helium-neon laser; Wavelength 0.6328 μm Power 10 mW
2	Block forming the spatial structure of the optical probe	Optical collimator forming a parallel laser beam with a cross section 5 mm
3	Multichannel block forming the polarization structure of the optical probe	System for forming linear (0º; 90º; 45º) and right-circular polarization (linear polarizer (Achromatic True Zero-Order Waveplate) – quarter-wave plate (B + W Kaesemann XS-Pro Polarizer MRC Nano))
4	Object block	Microscopic coordinate node
5	Block forming microscopic images	Polarizing micro lens (Nikon CFI Achromat P, working distance – 30mm, focal distance - 50mm, NA – 0.1, magnification – 4x)
6	Block of multichannel polarization filtration	Transmission system linear (0º; 90º; 45º; 135º) right- and left-circularly polarized components of light oscillations
7	Block of sampling digital microscopic images	Digital CCD camera (The Imaging Source DMK 41AU02.AS, monochrome 1/2 "CCD, Sony ICX205AL (progressive scan); resolution - 1280x960, size light-sensitive area - 7600x6200mkm; sensitivity - 0,05 lx, dynamic range - 8 bit, SNR - 9 bit) by polarization microobjective 7 (Nikon CFI Achromat P, focal length - 30 mm, numerical aperture - 0.1 increase - 4x)
8	Block of computer processing of Muller matrix polarimetry	Definition of statistical points of 1st-4th orders
Source: Authors		

The experimental methodology of azimuth-invariant Muller-matrix polarization microscopy includes the following steps:

▪ For each of the representative samples of BT, Muller-matrix invariant (MMI) maps of polycrystalline component of histological sections of internal organs and blood films were experimentally determined;

▪ Values of the statistical moments in the range of 1st to 4th orders (*Sr, Dp, As, Ek*), which characterize the MMI size distributions for each individual sample of BT, were calculated;

▪ Average value and the error of the magnitude of each of the central statistical moments in range of 1st to 4th orders were determined within the control and two experimental groups of BT;

▪ For each of the central statistical moments in range of 1st to 4th orders, which characterize MMI distributions, statistical reliability of differentiation of MMI maps of the polycrystalline component of histological sections of BT of internal organs and blood films was established;

▪ within the principles of evidence-based medicine, the operational characteristics of the force of the azimuthal-invariant Muller-matrix polarization microscopy method were calculated: sensitivity, specificity, balanced accuracy.

Thus, the temporal changes in the statistical moments of MMI distribution of BT preparations of the human body were determined: brain, liver, adrenal glands, myocardium, and polycrystalline blood films for both experimental and control groups.

Histological sections of the brain

Table 1 summarizes the results of monitoring of temporal changes in the statistical structure of the Muller-matrix images of the polycrystalline component of histological sections of the brain of all three groups of deaths with different TSD.

Table 1: Temporal dynamics of change of statistical moments of the 3rd - 4th orders, characterizing distributions of size of MMI of histological sections of a brain (T, hours).

T, hours	6	8	10	16	>24
Options	Control Group (CHD)				
SM_3	0.36 ± 0.014	0.54 ± 0.21	0.77 ± 0.34	1.41 ± 0.64	1.36 ± 0.61
P	$p<0.05$				$p<0.05$
SM_4	1.36 ± 0.061	2.14 ± 0.195	2.92 ± 0.13	5.31 ± 0.22	5.22 ± 0.21
P	$p<0.05$				$p<0.05$
T, hours	6	8	10	16	>24
Options	Study group 1 (alcohol poisoning)				
SM_3	0.71 ± 0.034	1.06 ± 0.044	1.51 ± 0.071	2.62 ± 0.11	2.57 ± 0.11
P	$p<0.05$				$p<0.05$
SM_4	1.07 ± 0.052	1.68 ± 0.73	2.27 ± 0.105	4.08 ± 0.18	3.99 ± 0.17
P	$p<0.05$				$p<0.05$
T, hours	6	8	10	16	>24
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	1.23 ± 0.059	1.88 ± 0.087	2.57 ± 0.11	4.33 ± 0.19	4.28 ± 0.19
P	$p<0.05$				$p<0.05$
SM_4	0.86 ± 0.042	1.44 ± 0.065	2.07 ± 0.094	3.99 ± 0.17	3.87 ± 0.17
P	$p<0.05$				$p<0.05$

Source: Authors

Table 2: Temporal dynamics of change of statistical moments of the 3rd - 4th orders, characterizing distributions of size of MMI of polycrystalline blood films (T, hours).

T, hours	6	8	10	18	>24
Options	Control Group (CHD)				
SM_3	0.11 ± 0.004	0.16 ± 0.007	0.21 ± 0.009	0.38 ± 0.016	0.36 ± 0.015
P	$p<0.05$				$p<0.05$
SM_4	0.095 ± 0.0049	0.15 ± 0.006	$0,21 \pm 0,009$	$0,44 \pm 0,018$	0.42 ± 0.017
P	$p<0.05$				$p<0.05$
T, hours	6	8	10	18	>24
Options	Study group 1 (alcohol poisoning)				
SM_3	0.21 ± 0.009	0.29 ± 0.012	0.38 ± 0.016	0.71 ± 0.031	0.68 ± 0.029
P	$p<0.05$				$p<0.05$
SM_4	0.19 ± 0.011	0.31 ± 0.014	0.43 ± 0.018	0.91 ± 0.042	0.88 ± 0.039
P	$p<0.05$				$p<0.05$
T, hours	6	8	10	18	>24
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	0.61 ± 0.031	0.92 ± 0.041	1.24 ± 0.052	2.14 ± 0.099	2.08 ± 0.098
P	$p<0.05$				$p<0.05$
SM_4	1.11 ± 0.094	1.74 ± 0.078	2.39 ± 0.105	4.21 ± 0.19	4.18 ± 0.19
P	$p<0.05$				$p<0.05$

Source: Authors

The obtained results show that the linear range of change in the magnitude of the central statistical moments is in the range of 1 4 order, which characterize the asymmetry and excess of the MMI size distribution of histological sections of the brain of the deceased, with the largest TSD being 18 hours.

- Value of the statistical moment of the 3rd order (asymmetry) varies within the following range:
  - Group 1: 0.36 to 1.41;
  - Group 2: 0.71 to 2.62;
  - Group 3: 1.23 to 4.33;
- Value of the statistical moment of the 4th order (excess) varies within the following range:
  - Group 1: 1.36 to 5.31;
  - Group 2: 1.07 to 4.08;
  - Group 3: 0.86 to 3.99.

Polycrystalline blood films

Table 2 summarizes the results of monitoring of temporal dynamics of necrotic changes in the statistical structure of the Muller map-matrix images of the optically anisotropic component of polycrystalline blood films of all three groups of deaths with different TSD.

The obtained results highlight that the linear range of change in the magnitude of the central statistical moments is between 1 to 4 order, which characterize the asymmetry and excess of the MMI size distribution of the polycrystalline blood films of the deceased, with the largest TSD being18 hours:

- Value of the statistical moment of the 3rd order (asymmetry) varies within the following range:
  - Group 1: 0.11 to 0.38;
  - Group 2: 0.21 to 0.71;
  - Group 3: 0.61 to 2.14;
- Value of the statistical moment of the 4th order (excess) varies within the following range:
  - Group 1: 0.095 to 0.44;
  - Group 2: 0.19 to 0.91;
  - Group 3: 1.11 to 4.21.

Histological sections of the liver

Table 3 summarizes the results of the monitoring of temporal dynamics of necrotic change in the coordinate structure of the Muller-matrix images of the polycrystalline component of the histological sections of the liver of all three groups of deaths with different TSD.

Table 3: Temporal dynamics of change of statistical moments of the 3rd, 4th orders, characterizing distributions of size of MMI of the histological sections of the liver (T, hours).

T, hours	6	8	14	20	>24
Options	Control Group (CHD)				
SM_3	0.71 ± 0.034	0.89 ± 0.041	1.38 ± 0.059	1.98 ± 0.088	1.91 ± 0.088
P	$p<0.05$				$p<0.05$
SM_4	0.54 ± 0.025	0.72 ± 0.031	1.28 ± 0.055	1.67 ± 0.073	1.61 ± 0.071
P	$p<0.05$				$p<0.05$
T, hours	6	8	14	20	>24
Options	Study group 1 (alcohol poisoning)				
SM_3	1.21 ± 0.062	1.49 ± 0.069	2.32 ± 0.105	3.88 ± 0.17	3.79 ± 0.16
P	$p<0.05$				$p<0.05$
SM_4	0.89 ± 0.043	1.11 ± 0.049	1.79 ± 0.076	2.81 ± 0.12	2.77 ± 0.12
P	$p<0.05$				$p<0.05$
T, hours	6	8	14	20	>24
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	1.85 ± 0.081	2.24 ± 0.099	3.47 ± 0.15	4.66 ± 0.21	4.58 ± 0.22
P	$p<0.05$				$p<0.05$
SM_4	1.43 ± 0.067	1.81 ± 0.088	3.03 ± 0.13	4.12 ± 0.18	4.07 ± 0.17
P	$p<0.05$				$p<0.05$
Source: Authors					

Here, the linear range of change in the magnitude of the central statistical moments is between 1st – 4th orders, which characterize the asymmetry and excess of the MMI size distribution of the histological sections of the liver of the deceased, with the largest TSD being 20 hours;

- Value of the statistical moment of the 3rd order (asymmetry) varies within the following range:
  - Group 1: 0.71 to 1.98;
  - Group 2: 1.21 to 3.88;
  - Group 3: 1.85 to 4.66;
- Value of the statistical moment of the 4th order (excess) varies within the following range:
  - Group 1: 0.54 to 1.67;
  - Group 2: 0.89 to 2.81;
  - Group 3: 1.43 to 4.12.

Histological sections of the adrenal glands

Table 4 illustrates the results of monitoring of temporal dynamics of necrotic change in the coordinate structure of the Muller-matrix images of the polycrystalline component of histological sections of the adrenal glands of all three groups of dead with different TSD.

Table 4: Temporal dynamics of change of statistical moments of the 3rd, 4th orders, characterizing distributions of size of MMI of histological sections of the adrenal glands (T, hours).

T, hours	6	8	14	20	>24
Options	Control Group (CHD)				
SM_3	0.31 ± 0.014	0.36 ± 0.014	0.49 ± 0.022	0.64 ± 0.027	0.61 ± 0.026
P	$p<0.05$				$p<0.05$
SM_4	0.26 ± 0.013	0.37 ± 0.016	0.59 ± 0.021	0.82 ± 0.035	0.79 ± 0.033
P	$p<0.05$				$p<0.05$
T, hours	6	8	14	20	>24
Options	Study group 1 (alcohol poisoning)				
SM_3	0.62 ± 0.031	0.74 ± 0.032	1.07 ± 0.044	1.42 ± 0.068	1.39 ± 0.069
P	$p<0.05$				$p<0.05$
SM_4	0.81 ± 0.042	1.03 ± 0.043	1.72 ± 0.078	2.39 ± 0.11	2.26 ± 0.105
P	$p<0.05$				$p<0.05$
T, hours	6	8	14	20	>24
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	0.51 ± 0.024	0.63 ± 0.027	0.99 ± 0.042	1.49 ± 0.067	1.44 ± 0.065
P	$p<0.05$				$p<0.05$
SM_4	0.74 ± 0.031	0.98 ± 0.043	1.73 ± 0.077	2.49 ± 0.11	2.38 ± 0.11
P	$p<0.05$				$p<0.05$

Source: Authors

The linear range of change in the magnitude of the central statistical moments of 1st– 4th orders, which characterize the asymmetry and excess of the MMI size distribution of histological sections of the adrenal glands of the deceased, with the largest TSD being 20 hours.

- Value of the statistical moment of the 3rd order (asymmetry) varies within the following range:
  - Group 1: 0.31 to 0.64;
  - Group 2: 0.62 to 1.42;
  - Group 3: 0.51 to 1.49;
- Value of the statistical moment of the 4th order (excess) varies within the following range:
  - Group 1: 0.26 to 0.82;
  - Group 2: 0.81 to 2.39;
  - Group 3: 0.74 to 2.49.

Histological sections of the myocardium

Table 5 illustrates the results of the monitoring of temporal dynamics of necrotic change in the coordinate structure of the Muller-matrix images of polycrystalline component of histological sections of the myocardium of all three groups of dead with different TSD.

The results show that the linear range of change in the magnitude of the central statistical moments is between 1st to 4th orders, which characterize the asymmetry and excess of the MMI size distribution of histological sections of the myocardium of the deceased, with the largest TSD is 24 hours.

Table 5: Temporal dynamics of change of statistical moments of the 3rd.4th orders, characterizing distributions of size of MMI of histological sections of the myocardium (T, hours).

T, hours	6	8	12	24	>36
Options	Control Group (CHD)				
SM_3	0.93 ± 0.046	1.01 ± 0.043	1.15 ± 0.049	1.56 ± 0.063	1.51 ± 0.062
P	$p<0.05$				$p<0.05$
SM_4	0.66 ± 0.031	0.74 ± 0.031	0.85 ± 0.039	1.28 ± 0.055	1.21 ± 0.054
P	$p<0.05$				$p<0.05$
T, hours	6	8	12	24	>36
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	1.38 ± 0.065	1.51 ± 0.068	1.77 ± 0.072	2.23 ± 0.098	2.18 ± 0.096
P	$p<0.05$				$p<0.05$
SM_4	0.95 ± 0.045	1.03 ± 0.041	1.24 ± 0.053	2.06 ± 0.094	2.01 ± 0.091
P	$p<0.05$				$p<0.05$
T, hours	6	8	12	24	>36
Options	Study group 2 (carbon monoxide poisoning)				
SM_3	2.03 ± 0.11	2.27 ± 0.105	2.68 ± 0.11	3.51 ± 0.15	3.47 ± 0.15
P	$p<0.05$				$p<0.05$
SM_4	1.81 ± 0.092	2.01 ± 0.097	2.44 ± 0.11	3.33 ± 0.14	3.24 ± 0.14
P	$p<0.05$				$p<0.05$

Source: Authors

- Value of the central statistical moment of 3rd order (asymmetry) varies within the following range:
  - Group 1: 0.93 to 1.56;
  - Group 2: 1.38 to 2.23;
  - Group 3: 2.03 to 3.51;
- Value of the central statistical moment of the 4th order (excess) varies within the following range:
  - Group 1: 0.66 to 1.28;
  - Group 2: 0.95 to 2.06;
  - Group 3: 1.81 to 3.33.

Accordingly, by azimuth-invariant Muller-matrix mapping of microscopic images of BT and body fluids, time intervals of determination of TSD for control and experimental groups were obtained. (Table 6)

Table 6: TSD time intervals

Muller-matrix mapping	
Histological section of the brain	16 hours
Polycrystalline film of blood	18 hours
Histological section of the liver	20 hours
Histological section of the adrenal glands	20 hours
Histological section of the myocardium	24 hours

Source: Authors

The results obtained confirmed the linear dependence of morphological changes in BT and human blood samples from the time of death, and their differential features depending on the cause of death.

Conclusion

A comprehensive experimental study of the diagnostic efficiency of the azimuthal-invariant Muller-matrix polarimetric microscopy of the polycrystalline component of histological sections of the brain, myocardium, adrenal glands, liver and polycrystalline blood films of dead persons for the diagnosis of TSD in cases of alcohol and carbon monoxide poisoning. A sensitivity range of 24 hours was determined from the method of the azimuthal-invariant Muller-matrix polarimetric microscopy of the polycrystalline component of histological sections of the myocardium for the diagnosis of TSD in cases of alcohol and carbon monoxide poisoning. The results revealed the effectiveness of the studied methodology and the prospect of further research in this direction.

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