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Diffuse tomography of human organ samples to establish the postmortem interval in alcohol and carbon monoxide poisoning

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ABSTRACT

Establishing of time interval that has elapsed since the death of a person has been and continues to be one of the most important issues to be addressed by a forensic expert during the autopsy. The main issue is to investigate and substantiate the information capabilities of the objective method of diffuse tomography of fluctuations of circular birefringence of histological sections of biological tissues (BT) in the diagnosis of post-mortem interval (PMI) in alcohol and carbon monoxide poisoning and also the possibility of their differentiation. The object of the study was histological sections of the brain, liver, adrenal glands, myocardium and polycrystalline blood films, selected from 150 corpses of both sexes, aged 20 to 68 years, with previously known time since death (TSD), ranging from 1 to 70 hours. The task of developing objective digital criteria for forensic diagnostics of PMI in alcohol and CO poisoning is based on the fluctuation of circular birefringence (FCB) of the polycrystalline component of humans BT. According to the results, a statistically significant possibility of differentiation of cases of alcohol and CO poisoning and TSD determination in the range of 20 to 48 hours (up to 0.5 hours) for the method of FCB of polycrystalline component of histological sections of adrenal glands.

Keywords: post-mortem interval, carbon monoxide poisoning, alcoholpoisoning, polarization, laser polarimetry

1. INTRODUCTION

Accurate calculation of TSD persist the most important issues to be determined by a forensic expert at autopsy or at the place of death. Among the scientific articles there is a great variety of methods to address this issue, based on the assessment of the dynamics of early and late postmortem changes (Maile et al., 2017; Gonnade et al., 2018; Olar et al., 2019). However, their accuracy varies within a few hours, which is not satisfactory for forensic investigators. It also must be considered that in this case it will not be possible

to reproduce the result obtained, that is, the result depends entirely on the experience and level of training of the expert conducting the research. In addition to determining of the post-mortem interval, an expert should diagnose the cause of death. However, there is currently a lack of information on specific morphological changes in BT in pathological conditions such as alcohol and carbon monoxide poisoning (Rose et al., 2017). Therefore, efforts should be directed to the development and implementation of new methods for estimating the PMI and death cause.

In forensic practice, laser polarimeter methods of microscopic examination of optically inhomogeneous biological structures using statistical analysis of digital polarization maps have successfully proven themselves. The School of Scientists, led by Professor V. Bachinsky and O. Ushenko proved that these methods provide the most complete information on the polycrystalline structure of BT and fluids of the human body and allow to study the dynamics of changes in different pathological conditions for the diagnosis of the PMI (Bachinskyi et al., 2018; Olar et al., 2019; Bachinskyi et al., 2017).

2. METHODS

The study was conducted in September 2021 - April 2022. The object of the study was histological sections of the brain, liver, adrenal glands, myocardium and polycrystalline blood films, selected from 150 corpses of both sexes, aged 20 to 68 years, with previously known TSD, ranging from 1 to 70 hours. All samples were divided into two experimental groups by cause of death: due to ethanol poisoning (1 group) and carbon monoxide (2 groups). BT samples from the dead persons due to IHD were used for control (control group, n=30). The basis of the task of developing objective digital criteria for forensic diagnostics of PMI in cases of ethanol and CO poisoning is based on establishing the dependences of the FCB of polycrystalline component of internal organs and human blood. The research methodology is illustrated in table 1. The obtained results were processed according to standard algorithms of MATLAB and Statistica software products.

Table 1 Scheme of the method of FCB of crystallization of biological layers

1	Source	Helium-neon laser (wavelength 0,6328 μm, power 10 mW)
2	The 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	The system of formation right-circular polarization (quarter-wave plate (B + Wkaesemann XS-ProPolarizer MRCNano))
4	Object block	Microscopic samples
5	Block formation of microscopic images	Polarizing microlens (Nikon CFI Achromat P, working distance – 30mm, focal distance - 50mm, NA – 0.1, magnification – 4x)
6	Block of multichannel polarization filtration	The transmission system linear (0^o; 90^o; 45^o; 135^o) right- and left-circularly polarized components of light oscillations
7	Block of sampling digital microscopic images	Digital CCD camera (CCD, Sony ICX205AL (progressive scan); polarization micro objective 7 (Nikon CFI Achromat P, focal length - 30 mm, numerical aperture - 0.1 increase - 4x)
8	Block of computer processing	Definition of statistical points of 1-4 orders

3. RESULTS

At the first stage of the study, we established the possibility of differentiation of ethanol and CO poisoning. Figure 1 shows the diffuse tomograms of the distributions of the FCB of optically active molecular complexes of histological sections of the brain (HSB) from experimental and control groups.

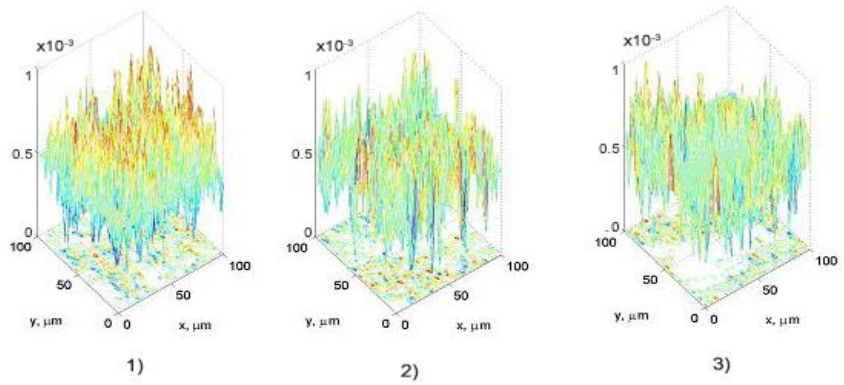

Figure 1 Maps of coordinate distributions of the value of FCB optical activity of HSBfrom control group (1), experimental group 1 (2) and experimental group 2 (3)

Calculations of the mean, variance, asymmetry and excess, which characterize the polarization-reproduced diffuse tomograms of the FCB were statistically significant (p_1; p_2; $p_{1,2}$ <0.05) for diagnostic use in forensic differentiation of representative samples of HSB from corpses that died of those who died of IHD and alcohol and CO poisoning (table 2). Figure 2 presents the results of differential mapping of molecular complexes (with optical activity) with algorithmic reproduction of coordinate distributions of FCB histological sections of the myocardial tissue (MT) from the experimental and two control groups of deaths from IHD, alcohol and CO poisoning.

Table 2 Statistical moments of the 1st - 4th orders, which characterize the distributions of the FCB value of samples of HSB

Sample	Brain		
Parameters	Control Group (n=30)	Study group 1 (alcohol poisoning) (n=60)	Study group 2 (CO poisoning) (n=60)
Mean, $SM_1 x10^{-2}$	0.43 ± 0,019	0.26 ± 0,012	0.11 ± 0,005
$p_1;p_2$		p_1<0.05	p_2<0.05
$p_{1;2}$		$p_{1,2}$<0.05	
Variance,$SM_2 x10^{-2}$	0.35 ± 0.016	0.24 ± 0.011	0.13 ± 0.006
$p_1;p_2$		p_1<0.05	p_2<0.05
$p_{1;2}$		$p_{1,2}$<0.05	
Asymmetry, SM_3	0.19 ± 0.009	0.28 ± 0.013	0.42 ± 0.019
$p_1;p_2$		p_1<0.05	p_2<0.05
$p_{1;2}$		$p_{1,2}$<0.05	
Excess, SM_4	0.27 ± 0.012	0.46 ± 0.023	0.79 ± 0.035
$p_1;p_2$		p_1<0.05	p_2<0.05
$p_{1;2}$		$p_{1,2}$<0.05	

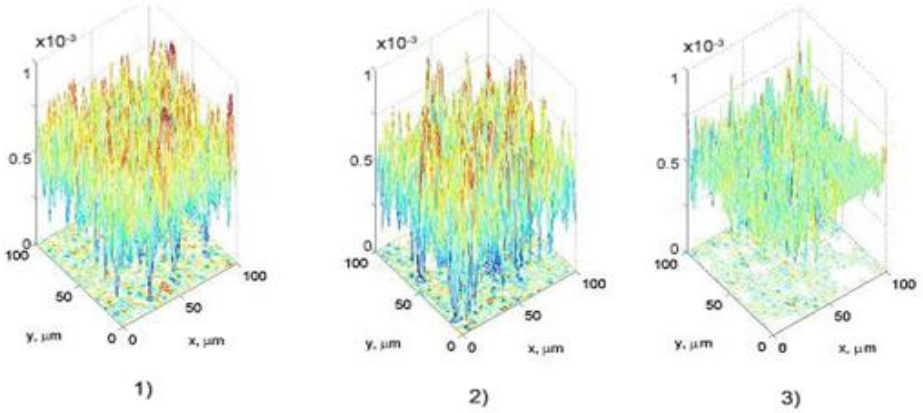

Figure 2 Maps of coordinate distributions of the value of FCB of histological samples of the MT from control group (1), experimental group 1 (2) and experimental group 2 (3)

Within the statistical access to the analysis of the structure of diffuse tomograms of the FCB it was found that the polarization manifestations of degenerative-dystrophic changes of structure of the MT correspond to a decrease in mean and variance, and an increase in asymmetry and excess died of alcohol and CO poisoning (table 4).

Table 4 Statistical moments of the 1st - 4th orders, which characterize the distributions of the value of FCB samples of histological sections of the MT from the control and experimental groups

Sample	Myocardium		
Parameters	Control Group (n=30)	Study group 1 (alcohol poisoning) (n=60)	Study group 2 (CO poisoning) (n=60)
Mean, SM_1x10^{-2}	0.24 ± 0.013	0.14 ± 0.006	0.08 ± 0.003
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Variance,SM_2x10^{-2}	0.28 ± 0.013	0.19 ± 0.008	0.11 ± 0.005
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Asymmetry, SM_3	0.14 ± 0.006	0.27 ± 0.012	0.35 ± 0.016
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Excess, SM_4	0.19 ± 0.009	0.35 ± 0.015	0.57 ± 0.026
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	

The diagnostic efficiency of statistically significant ($p_1;p_2;p_{1;2}<0.05$) differentiation of myocardial samples of deceased from all groups by calculation of a set of statistical moments of the 1st - 4th orders is demonstrated. Figure 3 shows the diffuse tomograms of the FCB of the structures of liver tissue (LT), and table 4 shows the values of $SM_{i=1,2,3,4}$ for all groups of samples.

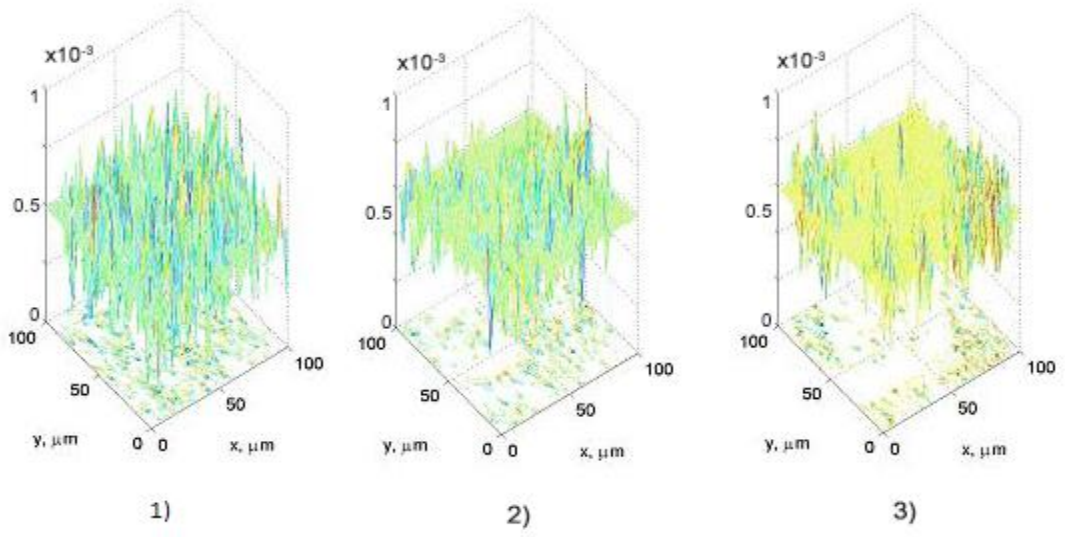

Figure 3 Distribution maps of the magnitude of fluctuations of the sections of the LT from control group (1), experimental group 1 (2) and group 2 (3).

Table 4 Statistical moments of the 1st - 4th orders of histological sections of the LT from the control and experimental groups

Sample	Liver		
Parameters	Control Group (n=30)	Study group 1 (alcohol poisoning) (n=60)	Study group 2 (CO poisoning) (n=60)
Mean, $SM_1 x10^{-2}$	0.06 ± 0.003	0.05 ± 0.003	0.045 ± 0.002
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Variance,$SM_2 x10^{-2}$	0.14 ± 0.007	0.11 ± 0.004	0.075 ± 0.003
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Asymmetry, SM_3	0.41 ± 0.022	0.69 ± 0.032	1.12 ± 0.058
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	
Excess, SM_4	0.64 ± 0.035	0.93 ± 0.045	2.23 ± 0.11
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1;2}<0.05$	

The results of diffuse tomography of the coordinate distributions of the FCD value of optically active molecular structures of samples of the adrenal glands (AG) are presented in Figure 4. The results revealed changes in tomographic manifestations of necrotic changes in the molecular structures of adrenal tissue for cases of alcohol and CO poisoning - a decrease in the magnitude and ranges of changes in FCB, which are quantified in decreasing SM_1 and SM_2 (mean and variance), and increasing SM_3 and SM_4 (excess of the distribution) (table 5). The study of the average values of $SM_{i=1,2,3,4}$ within the representative samples of samples from control and experimental samples of the A Grevealed statistical significance ($p_1;p_2;p_{1;2}<0.05$) in the differentiation of deaths from all groups.

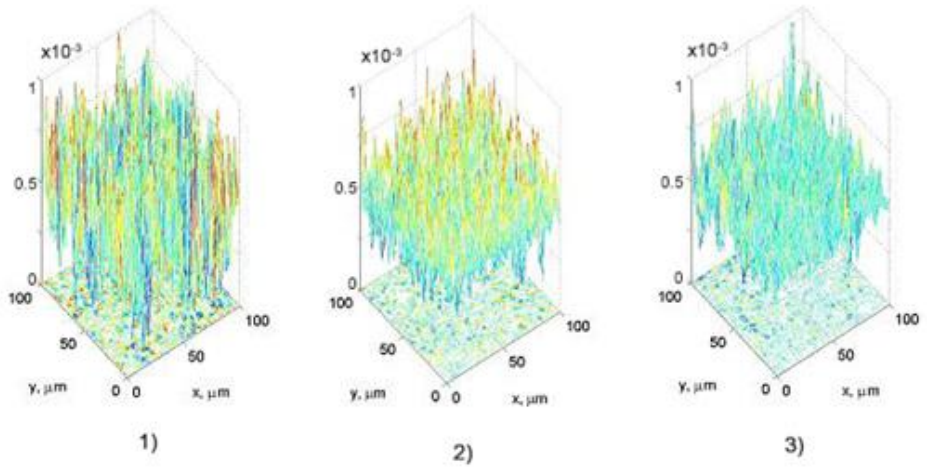

Figure 4 Diffuse tomograms of coordinate distributions of FCD in the samples of the AG from control group (1), experimental group 1 (2) and 2 (3).

Table 5 Statistical moments of the 1st - 4th orders, which characterize the coordinate distributions of the FCB value of samples of histological samples of the AG

Sample	Adrenal glands		
Parameters	Control Group (n=30)	Study group 1 (alcohol poisoning) (n=60)	Study group 2 (CO poisoning) (n=60)
Mean, SM_1x10^{-2}	0.28 ± 0.012	0.13 ± 0.006	0.065 ± 0.003
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1,2}<0.05$	
Variance,SM_2x10^{-2}	0.31 ± 0.014	0.15 ± 0.007	0.085 ± 0.003
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1,2}<0.05$	
Asymmetry, SM_3	0.31 ± 0.014	0.52 ± 0.024	0.89 ± 0.041
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1,2}<0.05$	
Excess, SM_4	0.39 ± 0.017	0.68 ± 0.033	0.97 ± 0.041
$p_1;p_2$		$p_1<0.05$	$p_2<0.05$
$p_{1;2}$		$p_{1,2}<0.05$	

Comparative analysis of diffuse tomograms of FCB revealed greater diagnostic sensitivity of the method of diffuse tomography of FCB optically active particles of AG samples to changes in ethanol and CO poisoning compared to similar tomographic studies of the brain, myocardium and liver. When calculating the balanced accuracy of statistical processing of the distributions of the FCB value of all groups of samples in the forensic differential diagnosis of ethanol and CO poisoning, the best results will be obtained for adrenal sections. Satisfactory (SM_2→82%) and excellent (SM_1,SM_3,SM_4→90-92%) level of values were established (table 6).

Table 6 Operational characteristics of the strength of the method of diffuse tomography FCB of samples of AG

Sample			Adrenal glands					
$SM_{i=1,2,3,4}$	SM_1		SM_2		SM_3		SM_4	
Se,%	a=37 b=8	82.2	a=37 b=8	82,2	a=41 b=4	91.1	a=42; b=3	93.3
Sp,%	c=37; d=8	82.2	c=37; d=8	82.2	c=40; d=5	88.8	c=40; d=5	88.8
Ac,%	n=150	92	n=150	82.2	n=150	90.45	n=150	91.05

The next stage of the research was the experimental testing of the method in establishing the TSD in casses of ethanol and CO poisoning. We will show the results on the example of adrenal tissue. Table 7 shows the data of the statistical analysis of the time monitoring of the necrotic change in the coordinate structure of the magnitude of FCB of the components of samples of the AG of all three groups with different PMI. The time duration (48 hours) of the linear range of changes in the magnitude of the SM_3 and SM_4 was determined by experimental studies using the method of diffuse tomography. The accuracy of TSD determination was 0.5 hours.

Table 7 Temporal dynamics of changes in the statistical moments of the 3rd - 4th orders of histological sections of the AG

T, hours	6	12	24	48	>60
Parameters	Control Group (n=30)				
SM_3	0.43 ± 0,021	0.67 ± 0,029	1.36 ± 0,065	2.88 ± 0,12	2.93 ± 0.13
p	*p<0.05*				*p>0.05*
SM_4	0.57 ± 0.027	0.99 ± 0.041	1.81 ± 0.086	3.67 ± 0.16	3.79 ± 0.17
p	*p<0.05*				*p>0.05*
T,hours	6	12	24	48	>60
Parameters	Study group 1 (alcohol poisoning) (n=60)				
SM_3	0.77 ± 0.034	1.25 ± 0.056	2.28 ± 0.105	4.19 ± 0.18	4.33 ± 0.19
p	*p<0.05*				*p>0.05*
SM_4	0.88 ± 0.039	1.41 ± 0.067	2.55 ± 0.11	4.21 ± 0.18	4.39 ± 0.19
p	*p<0.05*				*p>0.05*
T,hours	6	12	24	48	>60
Parameters	Study group 2 (CO poisoning) (n=60)				
SM_3	1.09 ± 0.046	1.71 ± 0.078	3.98 ± 0.18	5.77 ± 0.23	5.83 ± 0.24
p	*p<0.05*				*p>0.05*
SM_4	1.15 ± 0.055	2.07 ± 0.099	3.88 ± 0.17	7.41 ± 0.32	7.55 ± 0.33
p	*p<0.05*				*p>0.05*

We found the destruction of the polycrystalline structure of the AG of the dead with increasing observation time after death - the set of Mueller-matrix reproduced diffuse maps of the FCB is characterized by lower values of optical anisotropy fluctuations, indicating developed necrotic changes.

4. DISCUSSION

We have developed a new principle of studying the polycrystalline structure of BT - revealed the high efficiency of Mueller-matrix algorithmic reproduction of maps of FCB optically anisotropic component of tissues in differentiating the cause of death and establishing the PMI. Good results of application of Stokes polarimetric and multichannel Muller matrix mapping of digital microscopic images of BT by other scientists for using in forensic practice, including for establishment of TSD are described in literature sources (Bachinskiy et al., 2017; Ushenko et al., 2014; Prysyazhnyuk et al., 2016). Scientists have managed to achieve the precision of determining the TSD – 1.5 hours in the long range from 1-48 hours. However, we obtained a qualitatively new level of results of diffuse tomography in the differentiation of causes of death and determined PMI, which can be attributed on that this technique of multichannel reproduction of information about direct fluctuating polycrystalline structure, in contradistinction to the above methods necrotic changes in the polycrystalline structure with the exception of the distorting background of depolarization of laser radiation. In addition, in the postmortem period, fluctuations in optical anisotropy parameters persist even at the minimum level of birefringence.

The range of linear changes of SM of higher orders, and accordingly the analytical determination of TSD in comparison with indirect methods of polarization and Mueller matrix multichannel analysis of microscopic images for each type of BT and polarization tomography of birefringence maps increases 1.5 - 2.5 times. Accordingly, the detection range of TSD is improved and lies in the range from 24 to 48 hours. The accuracy of TSD determination is tripled to 0.5 hours.

5. CONCLUSION

Forensic efficiency of statistically significant differentiation of cases of alcohol and CO poisoning by the method of algorithmic reproduction of FCB polycrystalline component of samples of the brain, myocardium and adrenal glands was established. A sensitivity range of 20 to 48 hours was determined (with accuracy 0.5 hour) for the method of the FCB of the polycrystalline component of samples of the preparations of the adrenal glands for the diagnosis of PMI in cases of alcohol and CO poisoning. The results showed the effectiveness of the studied methodology and the prospect of further research in this direction.

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Author Contributions

Author contributed to the research and preparation of the manuscript.

Ethical approval

The study was approved by the Medical Ethics Committee of the Bukovinian State Medical University. (Protocol No.7 dated 26.05.2022).

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Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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