ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

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MULTI-PARAMETER MUELLER-MATRIX TOMOGRAPHY OF HISTOLOGICAL SAMPLES OF BIOLOGICAL TISSUES AS AN ACCURATE AND EFFECTIVE METHOD FOR DETERMINING THE DEGREE OF BLOOD LOSS

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Summary. Establishing the volume of blood loss is extremely important in the context of forensic practice, as it can indicate various circumstances of death and is important in solving criminal cases. Consideration of modern methods of determining this parameter in the article is relevant and aims to reveal new opportunities and perspectives in this field of forensic medical examination.

Aim of the work. To develop a set of new forensic criteria for accurate determination of the volume of blood loss using the method of multichannel polarization Muller-matrix tomography of histological sections of parenchymal organs and human blood samples.

Materials and methods. Samples of parenchymal organs and human blood were collected from 76 corpses of both sexes with varying degrees of blood loss from 0 mm³ to 2500 mm³. The research was carried out using the method of multichannel polarization Muller-matrix linear dichroism tomography of biological tissue samples.

Results. For all studied biological samples, a decrease in the level of circular birefringence (CB) of formed blood elements against the background of a gradual necrotic decrease in distributions of linear birefringence (LB) of the optical anisotropy of parenchymal tissues and blood films was established for the process of blood loss. The range of sensitivity of the method of differential Mueller-matrix tomography with algorithmic reproduction of linear dichroism maps to changes in blood loss volume of the deceased, which is (0 ± 2000) mm³, is determined.

Conclusions. The accuracy of the method of differential Mueller matrix mapping with algorithmic reproduction of linear dichroism maps of biological preparations was determined, which is 86-92 % in the range of the level of blood loss $\Delta V = (0\pm 2000)$ mm³.

Keywords: forensic medicine, blood loss, polarimetry, diagnostics, Mueller's matrix.

Introduction. In forensic medical practice, determining the volume of blood loss is a key aspect for reconstructing the circumstances of death and establishing its cause, which makes it possible to make a significant contribution to the investigation of the case [1-3].

When determining the causes of death from blood loss, experts often have to deal with issues related to the causes of blood loss, its volume, severity of damage and the role of this factor in the dying process. When investigating such cases, the police turn to experts to find out about the possibility of active actions of dying persons, to determine the effectiveness of medical assistance. Establishing these aspects is often a challenge for experts. In many cases, the circumstances of death remain unknown or there are no medical documents that could help in retrospective analysis. At the same time, determining the volume of blood loss through morphometric analysis during autopsy is not sufficiently accurate and objective, nor is it sufficient to resolve issues related to the speed of development of this process [4, 5].

Modern methods, approaches and technologies contribute to improving the accuracy and objectivity of determining the amount of blood loss, which has a significant practical value for forensic

experts and investigations of fatal cases. With the development of new technologies and analytical methods, which are aimed at the accurate determination of this indicator, the possibility of improving forensic investigations opens up [6, 7].

In this paper, we propose to consider the possibilities of multiparameter differential Mullermatrix tomography of linear dichroism of histological sections of parenchymal biological tissues and human blood samples in determining the volume of blood loss [8].

Aim of the work. To develop a set of new forensic medical criteria for accurate determination of the volume of blood loss using the method of multichannel polarization Muller-matrix tomography of histological sections of parenchymal organs and human blood samples.

Materials and methods. Samples of parenchymal organs and human blood were collected from 76 cadavers of both sexes aged 18 to 56 years with various degrees of blood loss.

Depending on the volume of blood loss (V), the following groups of samples were considered:

- $V = 0 \text{ mm}^3 \text{group } 1 \text{control group (10 samples);}$
- $V = (500 \pm 100) \text{ mm}^3 \text{group } 2 \text{ (6 samples)};$
- $V = (1000 \pm 100) \text{ mm}^3 \text{group 3 (12 samples)};$
- $V = (1500 \pm 100) \text{ mm}^3 \text{group 4} (13 \text{ samples});$
- $V = (2000 \pm 100) \text{ mm}^3 \text{group 5 (17 samples)};$
- $V = (2500 \pm 100) \text{ mm}^3 \text{group 6 (18 samples).}$

The research was carried out using a laser polarimeter of the following structure (fig. 1) [6-8]:



Fig. 1. Functional block diagram of multiparameter differential Mueller-matrix tomography of biological samples:

I – lighting unit (1 – laser; 2 – collimator);

II – polarization filter (3 – polarizer; 4 – quarter-wave phase plate);

III – object unit (5 – biological sample);

IV – projection unit (6 – polarizing microlens);

V-polarization analysis unit (7-polarizer; 8-quarter-wave phase plate);

VI – photoelectronic registration unit (9 – digital camera);

VII – data processing unit (10 – personal computer).

Statistical data processing was carried out using MS® Excel® 2010[™] and Statistica® 7.0 applications.

Results. Based on previous works, we believe that the process of blood loss leads to a decrease in the level of CB and dichroism of formed blood elements against the background of a gradual necrotic decrease in the distribution of LB and dichroism of optical anisotropy of fibrillar and parenchymal structures. Thus, the determination of the volume of lost blood is based on the establishment of relationships between changes in the distributions of parameters of various types of tomographically reproduced maps of phase and amplitude anisotropy and the level of blood loss of the deceased.

On a series of fragments of fig. 2 shows the experimentally determined coordinate distributions of random values of the dichroism value of the parenchymal structure of the histological sections of the spleen of the deceased from group 1 (1) and group 3.

(2).



Fig. 2. Maps and histograms of size distributions of histological sections of the spleen of the control (1) and experimental (2) groups.

Quantitatively, the scenario of changes in the distribution of the size of the LB of the parenchymal structure of the histological sections of the spleen of the deceased with different degrees of blood loss illustrate the significance of the statistical points listed in table 1.

Table 1

Statistical structure of linear birefringence maps of histological sections of the spleen o	f
deceased persons with various degrees of blood loss	

Blood loss, mm ³	0	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³
Average (SM_1)	0,16±0,007	$0,145\pm0,006$	0,12±0,005	0,099±0,005	$0,082{\pm}0,004$	0,095±0,006
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Dispersion (SM_2)	0,13±0,0064	$0,097{\pm}0,004$	0,065±0,003	0,036±0,001	0,015±0,001	0,026±0,001
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Asymmetry (SM_3)	1,02±0,046	1,36±0,057	$1,74\pm0,074$	2,15±0,104	2,51±0,12	2,21±0,106
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Excess (SM ₄)	1,36±0,061	1,65±0,053	1,98±0,105	2,34±0,108	2,62±0,12	2,23±0,11
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05

Thus, the value of the set of statistical moments of the 1st-4th order changes, according to the volume of blood loss, it is (0±2000) mm³.

In fig. 3 presents graphic dependencies – diagrams of changes in the value of statistical moments $SM_{1;2;3;4}$, which characterize the distributions of the LB value of spleen samples due to blood loss for all groups of the deceased.

From the obtained results of the method of multichannel polarization Müller-matrix tomography (fig. 3), it can be seen that the dynamics of changes in the value of statistical moments $SM_{1;2;3;4}$, which characterize the distribution of LB values of histological sections of the spleen, change within the blood loss volume of (0±2000) mm³.



Fig. 3. Dependencies of the average (SM1), dispersion (SM2), asymmetry (SM3) and excess (SM4), which characterize linear birefringence maps of histological sections of the spleen for the deceased with different degrees of blood loss.

The statistical parameters most sensitive to changes in the degree of LB of optically anisotropic parenchymal structures of histological sections of the spleen are asymmetry and excess.

Maps and histograms of distributions of the value of LB of polycrystalline fibrillar networks of the structures of histological sections of the kidneys of the deceased from group 1 (1) and group 3 (2), which were obtained using multichannel differential Mueller matrix mapping (fig. 4).



Fig. 4. Maps and histograms of distributions of the value of LB of histological sections of the kidneys of the control (1) and experimental (2) groups of the deceased.

From the analysis of the obtained data, it was found that with an increase in the volume of blood loss and a corresponding decrease in the concentration of formed blood elements, the magnitude of the degree of linear dichroism of the optically anisotropic collagen nets of the kidney decreases (fig. 4). Statistically, this is manifested in a decrease in the average and variance values, which characterize the distributions of the LB value of histological sections of kidney tissue of all groups of the deceased.

Also, the scenario of increased blood loss is accompanied by inverse changes in the magnitude of the statistical moments SM_3 and SM_4 , which characterize the asymmetry and excess of the distributions of the corresponding coordinate maps of the LB of histological sections of the kidney of the deceased in the range of the volume of blood loss up to V = (2000±100) mm³.

The results of the statistical analysis of changes in the structure of LB maps of histological sections of the kidneys of the deceased with different degrees of blood loss illustrate the statistical points $SM_{1,2,3,4}$, the values of which are given in table 2.

Table 2

Blood loss, mm ³	0	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³
Average (SM ₁)	0,18±0,008	0,153±0,007	0,127±0,005	0,098±0,005	0,069±0,003	0,078±0,004
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Dispersion (SM ₂)	0,13±0,005	0,11±0,004	0,093±0,004	0,071±0,003	0,043±0,002	0,056±0,002
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Asymmetry (SM ₃)	1,14±0,046	1,38±0,056	1,61±0,073	1,81±0,087	2,09±0,095	1,84±0,085
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05
Excess (SM ₄)	1,49±0,069	1,88±0,77	2,38±0,11	2,74±0,12	3,03±0,14	2,82±0,13
p	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05

Statistical structure of linear birefringence maps of histological sections of the kidneys of deceased persons with various degrees of blood loss

In fig. 5 presents graphical dependences of changes in the values of statistical moments $SM_{1;2;3;4}$, which characterize the coordinate structure of distributions of random values of linear dichroism by optically anisotropic collagen networks of biological crystals of a representative set of samples of histological sections of the kidneys of the deceased from all groups according to the level of blood loss.

From the obtained data of the statistical analysis of the results of multi-parameter Mueller matrix tomography of changes in the optically anisotropic absorption of optically anisotropic grids of biological crystals, which is due to blood loss (fig. 5, table 2), it can be seen that the values of the average, dispersion, asymmetry and excess, which characterize the distributions of the LB value of histological kidney sections of the deceased, change within the blood loss volume of (0 ± 2000) mm³.

The next stage of our work was the study of linear dichroism of polycrystalline blood films of corpses with different degrees of blood loss (fig. 6).

We would like to note that for the blood film, the main factor in changing the coordinate polycrystalline structure is the optical anisotropy of the shaped elements that scatter (depolarize) laser radiation against the background of the unchanged structural anisotropy of polycrystalline albumin – globulin networks [9]. Therefore, with a decrease in the concentration of formed blood elements in cases of blood loss, the value of linear dichroism of LB also decreases.



Fig. 5. Dependencies of the value of the mean (SM1), variance (SM2), asymmetry (SM3) and kurtosis (SM4), which characterize the LB maps of histological sections of the kidneys of the deceased with different degrees of blood loss.



Fig. 6. Maps and histograms of distributions of LB values of polycrystalline blood films of the control (1) and experimental (2) groups.

The results of the statistical analysis of the specified changes in the optically anisotropic linear dichroism of the polycrystalline blood films illustrate the statistical moments of the 1st to 4th orders, which are listed in table 3.

Table 3

corpses with unrerent degrees of blood loss								
Blood loss, mm ³	0	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³		
Average (SM ₁)	0,21±0,009	0,183±0,008	0,152±0,007	0,121±0,005	0,092±0,005	0,063±0,003		
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05		
Dispersion (SM ₂)	0,19±0,008	0,17±0,007	0,145±0,006	0,124±0,005	0,103±0,004	0,084±0,003		
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05		
Asymmetry (SM ₃)	0,36±0,014	0,66±0,027	0,94±0,043	1,24±0,065	1,57±0,077	1,85±0,084		
р	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05		
Excess (SM ₄)	0,57±0,023	0,78±0,032	0,99±0,045	1,19±0,052	1,47±0,064	1,72±0,075		
p	<0,05	<0,05	<0,05	>0,05	>0,05	>0,05		

Statistical structure of maps of linear birefringence of polycrystalline blood films of the corpses with different degrees of blood loss

The following statistical parameters turned out to be most sensitive to such changes in the concentration of formed elements – statistical moments of the 3rd and 4th orders, which characterize the asymmetry and excess of the linear dichroism value distributions of the polycrystalline blood films of the corpses, which change within the blood loss volume of (0 ± 2000) mm³.

Thus, the analysis of the results of the study of the effectiveness of determining the degree of blood loss by the method of differential Mueller-matrix mapping of coordinate distributions of the linear dichroism value of histological sections of parenchymal (spleen, kidney) tissues and polycrystalline blood films revealed: for all studied biological preparations, the sensitivity range of the method is (0 ± 2000) mm³.

The results of determining the accuracy of the proposed method are illustrated in tables 4-6.

Table 4

3 								
Blood loss, mm ³	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³			
Average (SM ₁)	86	86	84	82	66			
Dispersion (SM ₂)	92	92	90	88	70			
Asymmetry (SM ₃)	78	76	72	70	66			
Excess (SM ₄)	82	82	80	76	72			

Accuracy of As (%) of determining the volume of blood loss (spleen)

Table 5

Accuracy of As (%) of	determining	the volume	of blood	loss ((kidnev)
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Blood loss, mm ³	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³
Average (SM ₁)	90	88	86	86	72
Dispersion (SM ₂)	94	92	92	92	76
Asymmetry (SM ₃)	92	90	88	86	74
Excess (SM ₄)	84	82	82	80	68

Table 6

Blood loss, mm ³	(500±100) mm ³	(1000±100) mm ³	(1500±100) mm ³	(2000±100) mm ³	(2500±100) mm ³
Average (SM ₁)	78	76	74	70	62
Dispersion (SM ₂)	84	82	80	80	68
Asymmetry (SM ₃)	92	92	90	88	74
Excess (SM ₄)	94	94	92	90	76

Accuracy of As (%) of determining the volume of blood loss (blood films)

The accuracy of the method of differential Mueller-matrix tomography with algorithmic reproduction of linear dichroism maps of the polycrystalline structure of biological samples varies within: $\Delta V = (0 \pm 2000) \text{ mm}^3 \leftrightarrow 86-92 \%;$

 $\Delta V = 2500 \text{ mm}^3 \leftrightarrow 56-68 \%.$

The maximum level is achieved for the following statistical parameters that characterize the linear dichroism maps of the following biological preparations:

spleen $-SM_2 \Leftrightarrow 88\% - 92\%$; •

kidney - $\begin{cases} \mathbf{SM}_{1} \Leftrightarrow 86\% - 90\%; \\ \mathbf{SM}_{2} \Leftrightarrow 92\% - 94\%; \end{cases}$

 $\begin{bmatrix} \mathbf{SM}_{3} \Leftrightarrow 86\% - 92\%; \\ \mathbf{SM}_{3} \Leftrightarrow 88\% - 92\%; \\ \mathbf{SM}_{4} \Leftrightarrow 90\% - 94\%. \end{bmatrix}$

Conclusions.

1. The dynamics of changes in the magnitude of statistical moments of the 1st to 4th orders, which characterize the distributions of LB of histological sections of parenchymal tissues, as well as polycrystalline films of the blood of the dead with different volumes of blood loss $\Delta V = (0 \pm 2500) \text{ mm}^3$, were studied $\Delta V = (0 \pm 2500) \text{ mm}^3$.

2. The values and ranges of changes in the accuracy of the method of differential Mueller matrix mapping with algorithmic reproduction of linear dichroism maps of biological preparations are determined $\Delta V = (0 \pm 2000) \text{ mm}^3 \leftrightarrow 86-92 \%$.

3. It is shown that the maximum level is reached for statistical moments that characterize the LB map:

- histological sections of the spleen asymmetry and excess $SM_{3.4} \leftrightarrow 86-88$ %; •
- polycrystalline films of blood dispersion $SM_2 \leftrightarrow 90-92$ %. •

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БАГАТОПАРАМЕТРИЧНА МЮЛЛЕР-МАТРИЧНА ТОМОГРАФІЯ ГІСТОЛОГІЧНИХ ЗРАЗКІВ БІОЛОГІЧНИХ ТКАНИН ЯК ТОЧНИЙ І ДІЄВИЙ МЕТОД У ВИЗНАЧЕННІ СТУПЕНЯ КРОВОВТРАТИ

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Резюме. Встановлення об'єму крововтрати надзвичайно важливе в контексті судової практики, оскільки може вказувати на різні обставини смерті та мати велике значення при вирішенні кримінальних справ. Розгляд сучасних методів визначення цього параметра в статті є актуальним і має на меті розкрити нові можливості та перспективи в даній галузі судовомедичної експертизи.

Мета роботи. Розроблення комплексу нових судово-медичних критеріїв для точного встановлення об'єму крововтрати за допомогою методу багатоканальної поляризаційної Мюллерматричної томографії гістологічних зрізів паренхіматозних органів і зразків крові людини.

Матеріали та методи. Забір зразків паренхіматозних органів і крові людини проводили від 76 трупів обох статей з різним ступенем крововтрати від 0 мм³ до 2500 мм³. Дослідження виконували, застосовуючи метод багатоканальної поляризаційної Мюллер-матричної томографії лінійного дихроїзму зразків біологічних тканин.

Результати. Для всіх досліджених біологічних препаратів було встановлене характерне для процесу крововтрати зниження рівня циркулярного двопроменезаломлення (ЦД) формених елементів крові на тлі поступового некротичного зменшення розподілів лінійного двопроменезаломлення (ЛД) оптичної анізотропії паренхіматозних тканин і плівок крові. Був визначений діапазон чутливості методу диференційної Мюллер-матричної томографії з алгоритмічним відтворенням мап лінійного дихроїзму до зміни об'єму крововтрати померлих, що складав (0±2000) мм³.

Висновки. Була визначена величина точності методу диференційного Мюллер-матричного картографування з алгоритмічним відтворенням мап лінійного дихроїзму біологічних препаратів, що становила 86-92 % на діапазоні рівня крововтрати $\Delta V = (0\pm 2000)$ мм³.

Ключові слова: судова медицина, крововтрата, поляриметрія, діагностика, матриця Мюллера.

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