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## DIAGNOSTICS OF THE DEGREE OF BLOOD LOSS BY THE METHOD OF STATISTICAL ANALYSIS OF MAPS OF OPTICAL ACTIVITY OF THE POLYCRYSTALLINE COMPONENT OF BIOLOGICAL TISSUES AND FLUIDS

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**Summary.** The research was conducted to create a reliable and accurate approach to determining the volume of blood loss, which will allow forensic experts to increase objectivity and accuracy in establishing the causes and circumstances of death, as well as assessing the degree of traumatic injuries, and will help ensure more reliable examination results in forensic practice.

**The aim of the work** is to develop and evaluate the effectiveness of a method for differential diagnosis of the degree of blood loss in corpses using statistical analysis of optical activity maps of the polycrystalline component of biological tissues and fluids.

**Material and methods.** The study material was brain, kidney and blood samples ( $n=124$ ) from corpses aged 18 to 60 years with a previously known blood loss volume from  $0\text{mm}^3$  to  $2500\text{mm}^3$ . The study was conducted using the method of multiparametric differential Muller matrix tomography, which allows detecting changes in the structure of polycrystalline elements of biological tissues at different degrees of blood loss. Calculation of digital values was performed using a laser polarimeter of a standard scheme, further statistical processing was performed using MS® Excel® 2010™ and Statistica® 7.0 software.

**Results.** For all studied biological samples, the method demonstrates high sensitivity in the range of blood loss volume from  $0\text{mm}^3$  to  $1500\text{mm}^3$  with an accuracy level of 86%-92%. Within the range of blood loss volume from  $2000\text{mm}^3$  to  $2500\text{mm}^3$  the accuracy of the method decreases to 56%-68%. The maximum level of accuracy is achieved for statistical parameters of kidney samples ( $SM_4 \leftrightarrow 86\%-92\%$ ), brain ( $SM_4 \leftrightarrow 86\%-90\%$ ) and blood films ( $SM_2 \leftrightarrow 90\%-92\%$ ), which characterize the Muller matrix maps of circular birefringence.

**Conclusions.** Based on the conducted study, the high efficiency of the multiparametric Muller matrix differential tomography method in determining the degree of blood loss within the volume of lost blood  $\Delta V = 0\text{mm}^3 \div 1500\text{mm}^3$  was established. The obtained results demonstrate that this method allows for an objective assessment of structural changes in biological tissues and fluids caused by blood loss. This makes it a unique tool for objectively determining the volume of blood loss.

**Keywords:** forensic medicine, blood loss, Muller matrix tomography, diagnostics.

**Introduction.** Determining the extent of blood loss is one of the key aspects of forensic medical examination during the investigation of the causes and circumstances of death. Massive blood loss can be both the direct cause of death and a concomitant factor that complicates the overall clinical picture of traumatic injuries [1, 2]. Assessing the volume of blood loss is crucial in conducting forensic medical examination of a corpse for the accurate interpretation of the mechanisms of injury and establishing the sequence of events. However, existing methods for determining blood loss, such as visual assessment or biochemical tests, do not always meet high standards of accuracy and can be subjective or depend on many factors. Often, due to postmortem changes, as well as in the case of external blood loss, it is quite difficult to establish their exact volume [2-4].

Modern methods, such as optical analysis of polycrystalline tissue structures, can solve this problem, as they make it possible to detect changes at the microscopic level that correspond to different degrees of blood loss. [5]. In our opinion, the use of optical technologies in forensic practice opens new opportunities for differential diagnostics, allowing us to obtain additional information about structural changes in tissues caused by blood loss [6-8].

The proposed method of statistical analysis of optical activity maps of the polycrystalline component of biological tissues and fluids can significantly increase the accuracy of diagnostics,

allowing to establish the volume of blood loss with high accuracy and sensitivity. This, in turn, will contribute to the objectivity of forensic medical examination and increase the reliability of the obtained results, which is of critical importance for ensuring justice.

**The aim of the work** is to develop and evaluate the effectiveness of a method for differential diagnosis of the degree of blood loss in corpses using statistical analysis of optical activity maps of the polycrystalline component of biological tissues and fluids.

**Material and methods.** The study material was brain, kidney and blood samples (n=124) from deceased persons aged 18 to 60 years. According to the previously known volume of blood loss, all samples were divided into the following groups: group 1 (control) – 0 mm<sup>3</sup> (n=10); group 2 – 500±100 mm<sup>3</sup> (n=14); group 3 – 1000±100 mm<sup>3</sup> (n=25); group 4 – 1500±100 mm<sup>3</sup> (n=36); group 5 – 2000±100 mm<sup>3</sup> (n=21); group 6 – 2500±100 mm<sup>3</sup> (n=18).

The chosen research method is multiparametric differential Muller matrix tomography, which is based on the principles of optical polarimetry and allows detecting changes in the structure of polycrystalline elements of biological tissues. Such changes can be a clear indicator of the volume of blood loss, as it leads to specific microscopic changes in the optical activity of tissues, especially in protein structures. Statistical analysis of optical activity provides quantitative data that reflects the level of blood loss, making it particularly valuable for forensic examination.

The calculation of digital values was performed using a laser polarimeter of a standard circuit, and further statistical processing was performed using software. MS® Excel® 2010™ and Statistica® 7.0.

The determination of the volume of blood loss was carried out according to the following algorithm:

$$V_x = \left( SM_*^i(V_*) - SM_1^i(V_1) \right) \times \left( (V_2 - V_1) / \left( SM_2^i(V_2) - SM_1^i(V_1) \right) \right) \quad (1)$$

Here:

$SM^j$  – one of a set of statistical parameters;

$\Delta V = (V_2 - V_1)$  – diagnostically relevant range of change in blood loss volume  $V$ ;

$SM_*^j$  – value of the statistical parameter  $Q$  of the image of a histological section of tissue or blood film of a deceased person with an unknown volume of blood loss;

$V_*$  – specific value of blood loss.

**Results.** During the study of brain tissue of cadavers who died due to acute blood loss, it was found that the distributions of the value of the algorithmically reproduced phase Muller matrix invariant (MMI) of circular birefringence (CB) with a higher level of blood loss are characterized by a smaller mean value and the range of dispersion of random values of the optical activity of CB in comparison with the experimental sample from control group 1 (Fig. 1).

The revealed data can be connected with the fact that phase modulation in points of parenchymal structure of brain tissue is mainly formed due to CB. This mechanism is determined by the influence of the “island” anisotropy of molecular protein complexes and the anisotropy of blood formed elements. Due to this, the change in the phase MMI of the optical activity of CB is mainly due to a decrease in the concentration of formed elements against the background of insignificant CB.

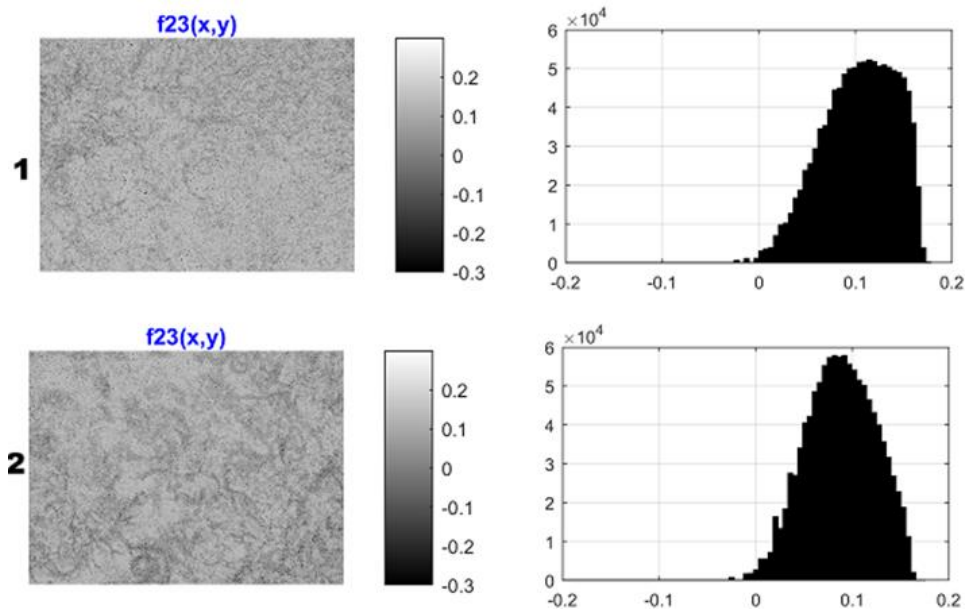


Fig. 1. Maps and histograms of CB values of histological brain sections of control (1) and experimental group 3 (2).

Quantitatively, this scenario of changes in the phase CB structure of samples of histological brain sections with different degrees of blood loss is illustrated by  $SM_{i=1;2;3;4}$ , which characterize the CB distributions and are presented in Table 1. A graphical representation of the change in their values is shown in Fig. 2, which demonstrates a linear change in the range of blood loss volume  $0 \text{ mm}^3 \div 1500 \text{ mm}^3$ .

Table 1

**Statistical structure of the Muller matrix invariant of circular birefringence maps of histological brain sections with different degrees of blood loss**

Blood loss, $\text{mm}^3$	0	$(500 \pm 100) \text{ mm}^3$	$(1000 \pm 100) \text{ mm}^3$
Average, $SM_1$	$0.164 \pm 0.0074$	$0.143 \pm 0.0061$	$0.124 \pm 0.0043$
$P$	$< 0,05$	$< 0,05$	$< 0,05$
Dispersion, $SM_2$	$0.16 \pm 0.0078$	$0.13 \pm 0.0057$	$0.103 \pm 0.0045$
$P$	$< 0,05$	$< 0,05$	$< 0,05$
Asymmetry, $SM_3$	$0.23 \pm 0.011$	$0.73 \pm 0.031$	$1.22 \pm 0.054$
$P$	$< 0,05$	$< 0,05$	$< 0,05$
Excess, $SM_4$	$0.38 \pm 0.015$	$0.98 \pm 0.042$	$1.52 \pm 0.067$
$P$	$< 0,05$	$< 0,05$	$< 0,05$
Blood loss, $\text{mm}^3$	$(1500 \pm 100) \text{ mm}^3$	$(2000 \pm 100) \text{ mm}^3$	$(2500 \pm 100) \text{ mm}^3$
Average, $SM_1$	$0.108 \pm 0.006$	$0.109 \pm 0.006$	$0.113 \pm 0.005$
$P$	$> 0,05$	$> 0,05$	$> 0,05$
Dispersion, $SM_2$	$0.069 \pm 0.004$	$0.075 \pm 0.005$	$0.071 \pm 0.004$
$P$	$> 0,05$	$> 0,05$	$> 0,05$
Asymmetry, $SM_3$	$1.74 \pm 0.089$	$1.83 \pm 0.092$	$1.79 \pm 0.081$
$P$	$> 0,05$	$> 0,05$	$> 0,05$
Excess, $SM_4$	$2.16 \pm 0.11$	$2.03 \pm 0.098$	$2.19 \pm 0.107$
$P$	$> 0,05$	$> 0,05$	$> 0,05$

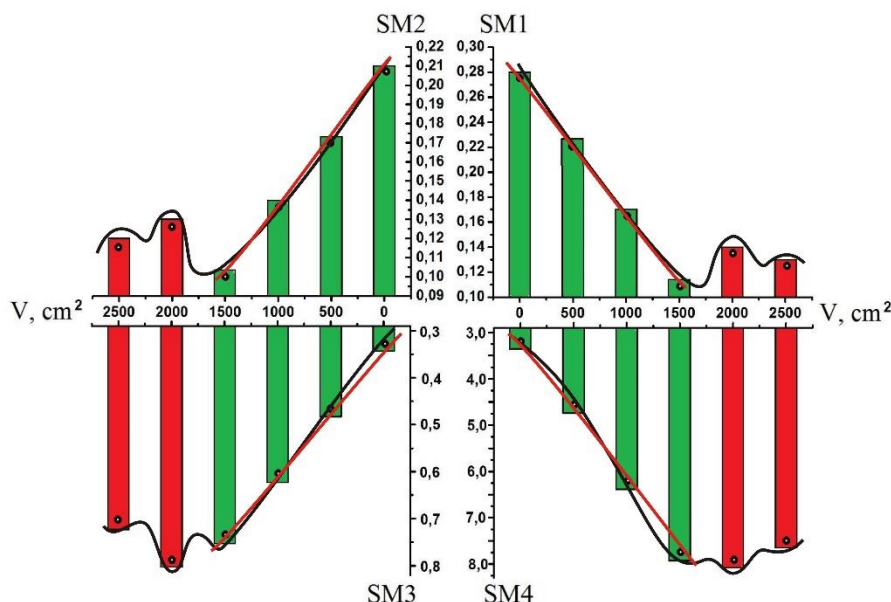


Fig. 2. Diagram of the values of average (SM<sub>1</sub>), dispersion (SM<sub>2</sub>), asymmetry (SM<sub>3</sub>) and excess (SM<sub>4</sub>), which characterize the MMI maps of CB of histological brain sections of deceased people with different degrees of blood loss.

Similar results were obtained when studying the polycrystalline component of histological sections of the kidney at different volumes of blood loss (Fig. 3). It was found that with an increase in the degree of blood loss (decrease in the concentration of formed blood elements), the depth of phase modulation of laser radiation by optically active molecular complexes of structurally anisotropic collagen networks of the kidney decreases.

Due to this, the average and dispersion values that characterize the distributions of the algorithmically reproduced phase MMI of CB of histological sections of kidney for all studied groups. This scenario is accompanied by inverse increasing changes in the magnitude of the 3rd and 4th order statistical moments, which characterize the distributions of the corresponding MMI of CB of histological sections of the kidney in the range of blood loss up to  $V = 1500\text{mm}^3 \pm 100\text{mm}^3$ .

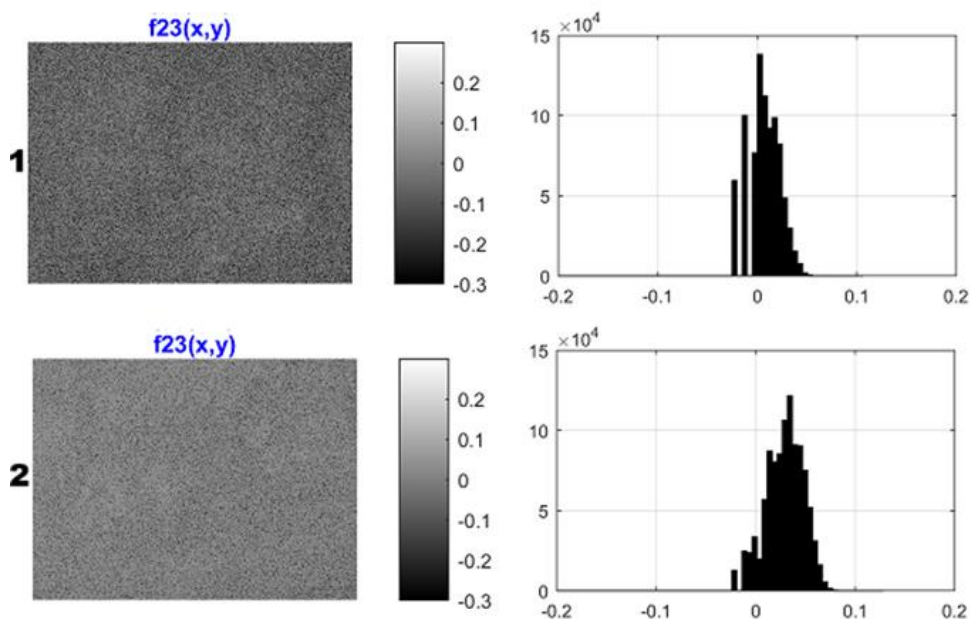


Fig. 3. Maps and histograms of CB values of histological kidney sections of control (1) and experimental group No. 3 (2) with different blood loss volumes.

The results of statistical analysis of such changes in the phase structure of CB of histological sections of the kidney with different degrees of blood loss illustrate  $SM_{i=1;2;3;4}$ , which are given in Table 2.

Table 2

**Statistical structure of the Muller matrix invariant of circular birefringence maps of histological sections of the kidney with different degrees of blood loss**

Blood loss, mm <sup>3</sup>	0	(500±100) mm <sup>3</sup>	(1000±100) mm <sup>3</sup>
Average, SM <sub>1</sub>	0.112±0.0057	0.092±0.0043	0.071±0.0034
<i>P</i>	<0,05	<0,05	<0,05
Dispersion, SM <sub>2</sub>	0.134±0.0061	0.112±0.0052	0.091±0.0044
<i>P</i>	<0,05	<0,05	<0,05
Asymmetry, SM <sub>3</sub>	0.83±0.041	1.17±0.054	1.42±0.065
<i>P</i>	<0,05	<0,05	<0,05
Excess, SM <sub>4</sub>	1.45±0.062	1.69±0.074	1.95±0.092
<i>P</i>	<0,05	<0,05	<0,05
Blood loss, mm <sup>3</sup>	(1500±100) mm <sup>3</sup>	(2000±100) mm <sup>3</sup>	(2500±100) mm <sup>3</sup>
Average, SM <sub>1</sub>	0.052±0.003	0.051±0.003	0.054±0.003
<i>P</i>	>0,05	>0,05	>0,05
Dispersion, SM <sub>2</sub>	0.072±0.0034	0.075±0.0035	0.078±0.0036
<i>P</i>	>0,05	>0,05	>0,05
Asymmetry, SM <sub>3</sub>	1.74±0.089	1.68±0.082	1.61±0.085
<i>P</i>	>0,05	>0,05	>0,05
Excess, SM <sub>4</sub>	2.02±0.098	1.93±0.091	1.89±0.089
<i>P</i>	>0,05	>0,05	>0,05

Fig. 4 presents diagrams of changes in the set of statistical moments  $SM_{i=1;2;3;4}$ , which characterize the coordinate structure of the phase MMI of the optical anisotropy of CB of histological sections of the kidney from all groups by the level of blood loss.

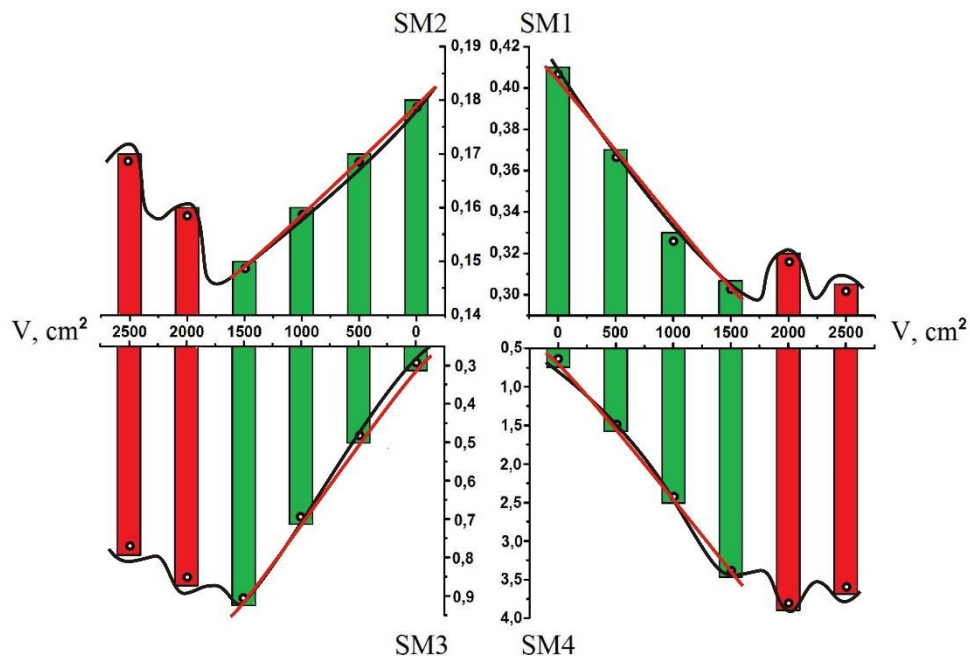


Fig. 4. Value of average (SM<sub>1</sub>), dispersion (SM<sub>2</sub>), asymmetry (SM<sub>3</sub>) and excess (SM<sub>4</sub>), for kidney samples from cadavers with varying degrees of blood loss.

From the obtained data of statistical analysis of polarization manifestations of changes in optical anisotropy caused by blood loss (Fig. 4, Table 2) it is seen that the values of the average, dispersion, asymmetry and excess, which characterize the distributions of the optical anisotropy of polycrystalline networks of histological sections of the kidney of the deceased, vary within the volume of blood loss 0 mm<sup>3</sup> ÷ 1500mm<sup>3</sup>. The most sensitive to changes in optical anisotropy of the morphological structure of the kidney at different levels of blood loss were asymmetry and excess, as

in the study of brain tissue.

At the next stage, we examined blood samples from individuals with different levels of blood loss. Multiparametric Muller matrix tomography allowed us to directly detect changes in the optical anisotropy of CB due to a decrease in the concentration of formed blood elements.

Figure 5 presents maps and histograms of the distributions of the phase MMI of CB values of samples of polycrystalline blood films from control group 1 (1) and experimental group 3 (2).

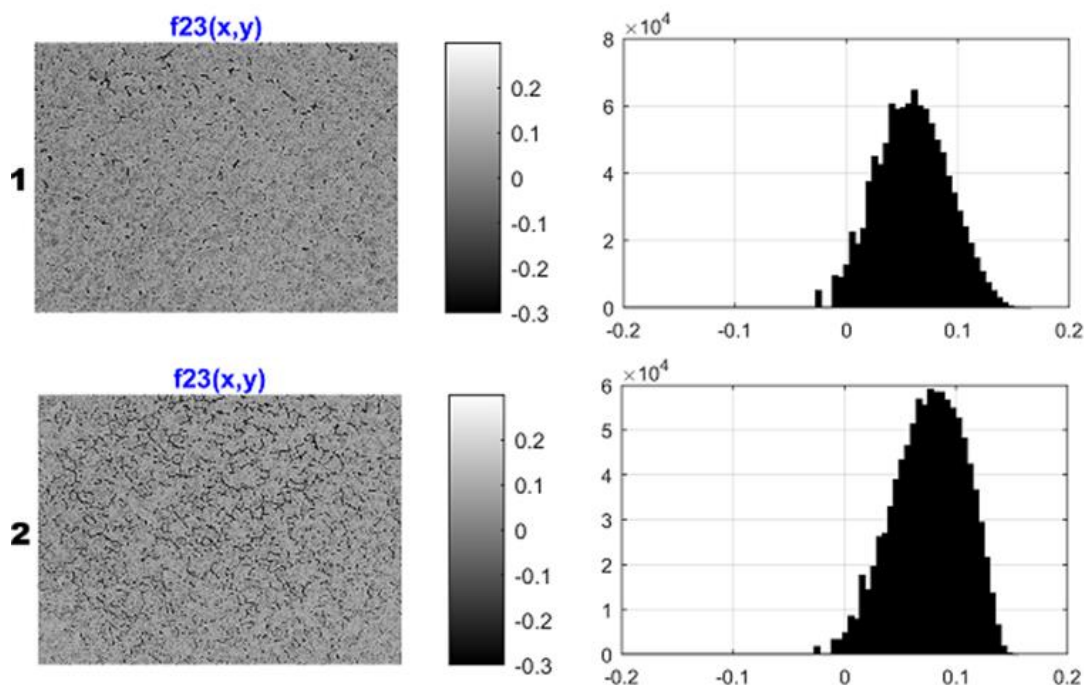


Fig. 5. Maps and histograms of the distribution of the phase MMI of CB values of blood films from the control (1) and experimental group 3 (2).

For blood, the main factor in changing the coordinate polycrystalline structure is the optical anisotropy of the formed elements, which scatter (depolarize) laser radiation against the background of the unchanged structural anisotropy of polycrystalline albumin-globulin networks. Therefore, with a decrease in the concentration of formed elements in blood in cases of blood loss, the depth of phase modulation of laser radiation by protein networks decreases. This, in turn, is illustrated by a decrease in the CB values of the optically active component of such samples (table 3).

Table 3

**Statistical structure of circular birefringence maps of polycrystalline blood films at different blood loss volumes**

Blood loss, mm <sup>3</sup>	0	(500±100) mm <sup>3</sup>	(1000±100) mm <sup>3</sup>
Average, SM <sub>1</sub>	0.153±0.0067	0.122±0.0053	0.091±0.0044
<i>p</i>	<0,05	<0,05	<0,05
Dispersion, SM <sub>2</sub>	0.131±0.0064	0.11±0.0051	0.097±0.0047
<i>p</i>	<0,05	<0,05	<0,05
Asymmetry, SM <sub>3</sub>	0.68±0.031	1.19±0.052	1.72±0.077
<i>p</i>	<0,05	<0,05	<0,05
Excess, SM <sub>4</sub>	0.84±0.039	1.22±0.064	1.65±0.059
<i>p</i>	<0,05	<0,05	<0,05
Blood loss, mm <sup>3</sup>	(1500±100) mm <sup>3</sup>	(2000±100) mm <sup>3</sup>	(2500±100) mm <sup>3</sup>
Average, SM <sub>1</sub>	0.062±0.003	0.058±0.003	0.059±0.003
<i>p</i>	>0,05	>0,05	>0,05
Dispersion, SM <sub>2</sub>	0.071±0.0034	0.073±0.0035	0.075±0.0036
<i>p</i>	>0,05	>0,05	>0,05
Asymmetry, SM <sub>3</sub>	2.17±0.099	2.08±0.092	2.16±0.095

<i>P</i>	>0,05	>0,05	>0,05
Excess, SM <sub>4</sub>	2.06±0.098	1.98±0.091	1.92±0.089
<i>P</i>	>0,05	>0,05	>0,05

Figure 6 presents graphical diagrams of changes in the set of statistical moments SM<sub>1;2;3;4</sub>, which characterize algorithmically reproduced Muller matrix maps of CB of polycrystalline blood films with different degrees of blood loss. The results of the study of the dynamics of changes (Fig. 6) in the magnitude of statistical values for polycrystalline blood films vary linearly within the range of blood loss volume 0 mm<sup>3</sup> ÷ 1500mm<sup>3</sup>.

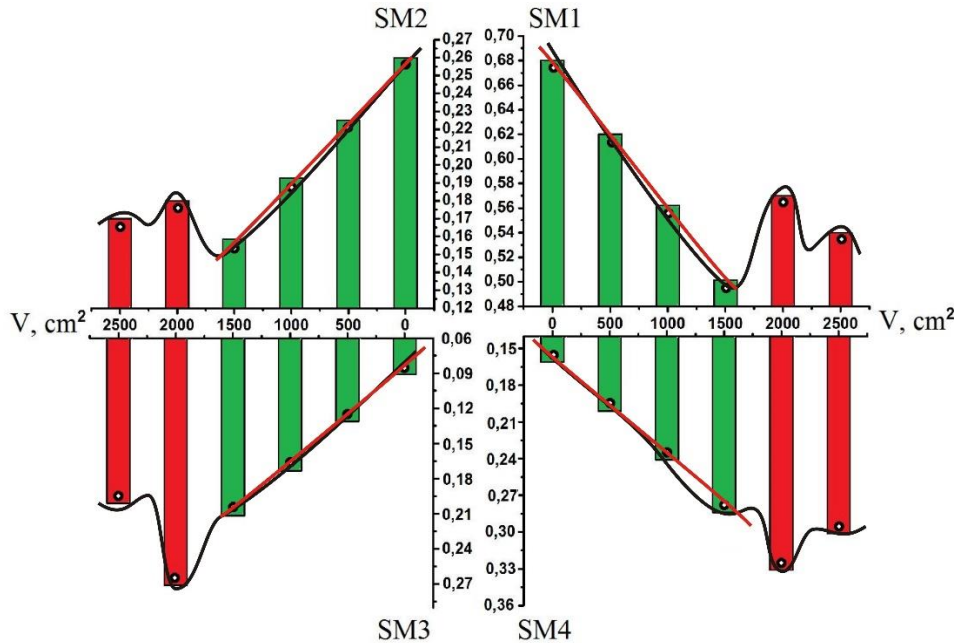


Fig. 6. Dependences of the values of average (SM<sub>1</sub>), dispersion (SM<sub>2</sub>), asymmetry (SM<sub>3</sub>) and excess (SM<sub>4</sub>), for blood samples of deceased persons with different degrees of blood loss.

We would like to present the systematic results of the study of the effectiveness of determining the volume of blood loss by the method of multichannel Muller matrix tomography of CB of histological sections of the kidney, brain and blood films from corpses with different volumes of blood loss. For each statistical moment that characterizes the MMI distributions of a set of biological tissue and blood samples from different study groups, the accuracy of determining the degree of blood loss was established based on experimentally obtained nomograms (Tab. 4-6).

Table 4

**Accuracy of blood loss determination for brain samples**

Blood loss volume, mm <sup>3</sup>	(500±100) mm <sup>3</sup>	(1000±100) mm <sup>3</sup>	(1500±100) mm <sup>3</sup>	(2000±100) mm <sup>3</sup>	(2500±100) mm <sup>3</sup>
SM <sub>1</sub>	84	82	80	56	53
SM <sub>2</sub>	82	80	80	58	56
SM <sub>3</sub>	88	86	84	63	62
SM <sub>4</sub>	90	88	86	62	64

Table 5

**Accuracy of blood loss determination for kidney samples**

Blood loss volume, mm <sup>3</sup>	(500±100) mm <sup>3</sup>	(1000±100) mm <sup>3</sup>	(1500±100) mm <sup>3</sup>	(2000±100) mm <sup>3</sup>	(2500±100) mm <sup>3</sup>
SM <sub>1</sub>	58	62	56	54	56
SM <sub>2</sub>	68	72	56	58	56
SM <sub>3</sub>	90	88	84	66	64
SM <sub>4</sub>	92	90	86	61	56

Table 6

**Accuracy of blood loss determination for blood samples**

Blood loss volume, mm <sup>3</sup>	(500±100) mm <sup>3</sup>	(1000±100) mm <sup>3</sup>	(1500±100) mm <sup>3</sup>	(2000±100) mm <sup>3</sup>	(2500±100) mm <sup>3</sup>
SM <sub>1</sub>	68	64	60	60	57
SM <sub>2</sub>	90	92	90	76	72
SM <sub>3</sub>	70	72	67	63	64
SM <sub>4</sub>	72	70	66	61	62

For all studied biological samples, the method demonstrates high sensitivity in the range of blood loss volume from 0 mm<sup>3</sup> to 1500 mm<sup>3</sup> with an accuracy level of 86-92%. Within the range of blood loss from 2000 mm<sup>3</sup> to 2500 mm<sup>3</sup> the accuracy of the method decreases to 56-68%, which indicates the dependence of diagnostic efficiency on the degree of blood loss.

• The maximum level is achieved for the following statistical parameters that characterize the Mueller matrix CB maps of the following biological samples:

- kidney -  $\{SM_3 \Leftrightarrow 84\% - 90\%;$   
 $SM_4 \Leftrightarrow 86\% - 92\%;$
- brain -  $SM_4 \Leftrightarrow 86\% - 90\%;$
- blood -  $\{SM_2 \Leftrightarrow 90\% - 92\%.$

**Conclusions.** Based on the conducted study, the high efficiency of the multiparametric Muller matrix differential tomography method in determining the degree of blood loss within the volume  $\Delta V = 0 \text{ mm}^3 \div 1500 \text{ mm}^3$ . The obtained results demonstrate that this method allows for an objective assessment of structural changes in biological tissues and fluids caused by blood loss. This makes it a unique tool for objectively determining the volume of blood loss.

The introduction of this method into forensic practice will contribute to increasing the accuracy and objectivity of examinations related to establishing the causes of death, especially in cases involving massive blood loss. This provides more reliable data for legal investigations and improves the quality of forensic conclusions.

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## ДІАГНОСТИКА СТУПЕНЯ КРОВОВТРАТИ МЕТОДОМ СТАТИСТИЧНОГО АНАЛІЗУ МАП ОПТИЧНОЇ АКТИВНОСТІ ПОЛКРИСТАЛІЧНОЇ СКЛАДОВОЇ БІОЛОГІЧНИХ ТКАНИН І РІДИН

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**Резюме.** Дослідження спрямоване на створення надійного та точного підходу для

визначення об'єму крововтрати, який дозволить судово-медичним експертам підвищити об'єктивність та точність у встановленні причин і обставин смерті, а також оцінці ступеня травматичних ушкоджень та допоможе забезпечити більш надійні результати експертизи в судовій практиці.

**Мета роботи** – розробити та оцінити ефективність методу диференціальної діагностики ступеня крововтрати у померлих за допомогою статистичного аналізу мап оптичної активності полікристалічної складової біологічних тканин і рідин.

**Матеріали та методи.** Матеріалом дослідження були зразки мозку, нирки та крові ( $n=124$ ) померлих віком від 18 до 60 років з попередньо відомим об'ємом крововтрати від  $0\text{mm}^3$  до  $2500\text{mm}^3$ . Дослідження проводили методом багатопараметричної диференціальної Мюллер-матричної томографії, що дозволяє виявляти зміни у структурі полікристалічних елементів біологічних тканин при різних ступенях крововтрати. Обчислення цифрових значень проводили за допомогою лазерного поляриметра стандартної схеми, подальшу статистичну обробку проводили використовуючи програмне забезпечення MS® Excel® 2010™ та Statistica® 7.0

**Результати дослідження.** Для всіх досліджених біологічних препаратів метод демонструє високу чутливість у діапазоні об'єму крововтрати від  $0\text{mm}^3$  до  $1500\text{mm}^3$  з рівнем точності 86%-92%. У межах об'єму крововтрати від  $2000\text{mm}^3$  до  $2500\text{mm}^3$  точність методу знижується до 56%-68%. Максимальний рівень точності досягається для статистичних параметрів препаратів нирки ( $SM_4 \leftrightarrow 86\%-92\%$ ), мозку ( $SM_4 \leftrightarrow 86\%-90\%$ ) та плівок крові ( $SM_2 \leftrightarrow 90\%-92\%$ ), які характеризують Мюллер-матричні мапи циркулярного двопронезаломлення.

**Висновки.** На основі проведеного дослідження було встановлено високу ефективність методу багатопараметричної Мюллер-матричної диференціальної томографії у визначенні ступеня крововтрати у померлих в межах об'єму втраченої крові  $\Delta V = 0\text{mm}^3 \div 1500\text{mm}^3$ . Отримані результати демонструють, що цей метод дозволяє об'єктивно оцінити структурні зміни в біологічних тканинах і рідинах, що спричинені втратою крові, що робить його унікальним інструментом для об'єктивного визначення об'єму крововтрати.

**Ключові слова:** судова медицина, крововтрата, Мюллер-матрична томографія, діагностика.

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