ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

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POSSIBILITIES OF DETERMINING THE TIME SINCE DEATH USING THE METHODS OF MULTIDIMENSIONAL POLARIZATION AND AUTOFLUORESCENCE MICROSCOPY OF THE HUMAN VITREOUS BODY

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Summary. Given the current state of affairs in practical forensic medicine and the considerable amount of scientific research on the exact determination of the postmortem interval, we conclude that the determination of the time since death is a key problem, but not yet sufficiently solved. Accurately estimating the time since death is critical to many investigations and remains one of the most challenging variables.

Carrying out an accurate determination of the time of death is important using a complex approach, since solving this problem requires taking into account all aspects of thanatogenesis, the influence of environmental factors and other circumstances of the case. This leads to an increase in the popularity of global research involving the latest advances in medicine, chemistry, physics and forensics. The use of comprehensive research allows us to come closer to establishing the approximate range of the time since death.

Aim of the work. Development of a complex of objective forensic medical criteria to improve the possibilities of accurately determining the time since death over a long period of time by using multidimensional polarization and autofluorescence microscopy of the human vitreous body.

Materials and methods. The object of the study were samples of human vitreous body, deceased from cardiovascular pathology, with a known time of death.

The following research methods were used: Mueller-matrix mapping, microscopic polarization tomography, spectral-selective laser-induced autofluorescence, statistical and wavelet analysis of the results of experimental data.

Results. The significant effectiveness of the proposed methods in accurately determining the time of death has been demonstrated. In particular, polarization microscopic tomography using scale-selective wavelet analysis of distributions of linear birefringence of human vitreousbody layers provides a diagnostic range for determining the time since death of 36 hours with an accuracy of up to 14-16 minutes, which was not achieved by any of the existing laser polarization methods. At the same time, the combination of polarization tomography of biological tissues with spectral-selective laser fluorescence microscopy – one of the most sensitive methods, due to the assessment of the concentration parameters of molecules, opens a new window of possibilities and establishing the time since death and diagnosis of various pathological conditions. The obtained results confirm the effectiveness of this technique and show the diagnostic range of determining the time since death of 36 hours with an accuracy of up to 15-20 minutes.

Conclusions. The innovative approach proposed in the scientific study makes a significant contribution to the development of forensic medical examination, providing more accurate and reliable identification of the time since death, which can be useful in criminal investigations and in solving other forensic tasks.

Keywords: forensic medicine, vitreous body, time since death, diagnosis.

Introduction. One of the key challenges in forensic medicine is the accurate determination of time since death (TSD) at various stages of postmortem morphological changes in human biological tissues. At the current stage, the modernization and development of new, more advanced criteria, methods and approaches to the determination of TSD remain an extremely urgent task in the field of forensic science and practice. Improving the accuracy and objectivity of the expert assessment of the TSD a significant practical effect, contributing to more effective investigations of crimes against the lives of citizens [1-4].

To determine the TSD, experts use various approaches, in particular, visual and descriptive analysis of early and late cadaveric phenomena, supravital reactions, as well as instrumental and laboratory methods, such as biochemical analysis, physical and mathematical models of changes in biological tissues after death [5]. However, traditional methods often do not achieve high accuracy and objectivity due to various limitations and shortcomings that may arise depending on the individual characteristics of the organism and environmental conditions [6, 7].

Therefore, in order to improve the determination of the TSD, scientists are looking for new ways, including the use of modern laboratory and instrumental methods that allow quantitative assessment of changes in biological tissues after death [5-7]. Such changes associated with various physical, metabolic, autolytic, physiological and biochemical processes that occur in the body after death alternately with the passage of time, which makes it possible to evaluate them and helps to make assumptions about the possible antiquity of the TSD. However, further research and improvement of these methods is necessary in order to increase the accuracy and objectivity of determining the TSD in forensic medical practice [8, 9].

In the world scientific literary sources, a number of scientific works are known, dedicated to the possibilities of objective biophysical monitoring of changes in the biochemical and polycrystalline structures of biological tissues.The implementation of these methods in forensic science and practice was carried out by Prof. V. T. Bachynskyi and scientists of his school [10-12]. Significant fundamental results were achieved [10], however, in our opinion, it is important to develop methods that would allow obtaining more accurate results in working with depolarizing and partially depolarizing tissues of the human body. In particular, it is necessary to expand the arsenal of experimental methods for the study of such objects based on differential Mueller-matrix mapping and autofluorescence microscopy of human tissue.

Aim of the work. Development of a complex of objective forensic medical criteria to improve the possibilities of accurately establishing the TSD over a long period of time by using multidimensional polarization and autofluorescence microscopy of the human vitreous body (VB).

Materials and methods. To establish the diagnostic possibilities of laser polarization-correlation and autofluorescence methods in the diagnosis of TSD, samples of the VB of corpses, who died of cardiovascular pathology with a known TSD were studied. Exclusion criteria were: presence of brain and eyeball injury, laboratory confirmed presence of any endogenous and exogenous intoxications.

The following research methods were used: Mueller-matrix mapping, microscopic polarization tomography, spectral-selective laser-induced autofluorescence, statistical and wavelet analysis of the results of experimental data.These forensic methods are based on the definition and diagnostic use of a set of objective relationships between the distributions of the obtained experimental data and the TSD.

Using the methods of polarization and spectrally selective fluorescence microscopy, a series of coordinate distributions of the information parameters of the set of experimental data for VB layers with different TSD was determined. The time elapsed since death was determined according to the following algorithm:

$$\tau_x(TSD) = \frac{SM_i^{(1)} - SM_i^{(0)}}{SM_i^{(2)} - SM_i^{(1)}}(\tau_2 - \tau_1)$$

Results. The obtained results show that the main factor in the formation of the coordinate structure of the parameter distributions of polarization microscopic images is the optical anisotropy of biological objects. This mechanism leads to phase shifts between the orthogonal components of the laser radiation amplitude at the points of the microscopic image of the VB layer.

Such phase distributions in the microscopic image are formed due to the influence of the following factors:

- structural anisotropy of a spatially ordered network of collagen fibrils – linear birefringence (LB);
- «partial» anisotropy of molecular protein complexes of the VB – circular birefringence (CB);
- multiple scattering of laser radiation in the volume of the optical anisotropicof the VB layer.

Due to the last factor, the distributions of the polarization parameters of microscopic images are averaged and the phase modulation is reduced in the plane of the microscopic image of the VB samples.

Based on this, it is possible to formulate the following scenario of the dynamics of the transformation of polarization manifestations of postmortem changes in the polycrystalline structure of the human VB layersat different TSD:

1) with an increase in TSD, the level of optical anisotropy of theVB decreases – the collagen network is disordered and the concentration of protein complexes decreases;
2) optically, this process of postmortem changes is manifested in a decrease in the depth of phase modulation of laser radiation by polycrystalline structures of the VB;
3) statistically, an increase in the degree of postmortem destruction of the polycrystalline component of the VB is accompanied by a decrease in the mean (SM_1) and variance (SM_2), and at the same time, a decrease in the level of LB and CB of VB leads to an increase in the value of asymmetry (SM_3) and excess(SM_4).

It is known that the disadvantage of Stokes-polarimetric and Mueller-matrix mapping, which were proposed in the works of other authors, is the poor reproducibility of the methods due to the azimuthal dependence of the azimuth value and the ellipticity of the polarization on the rotation of the sample relative to the direction of irradiation or changes in the state of polarization of the probing beam [8, 13].The mentioned techniques belong to the case of diagnosing the structure of optically thin biological layers that do not depolarize laser radiation, but only transform it. At the same time, it is practically impossible to achieve such an approach in the real practice of a forensic experiment. Since the VB belongs to partially depolarizing biological tissues, the best results were achieved by using polarization microscopic tomography (reconstruction) of human VB preparations with different TSD. This method providesobtaining diagnostically important relationships between the distributions of the average values of the anisotropy parameters of biological layers and its spatial transformations.

We will demonstrate the obtained results on the example of the LB of the crystalline component of human VB. Fig. 1 shows the distributions of the LB values of the VB layers of the deceased with TSD at 3 hours and 12 hours. The obtained results illustrate the existence of differences between the data of microscopic polarization tomography of the LB polycrystalline component of the VB layers of the deceased with different TSD.

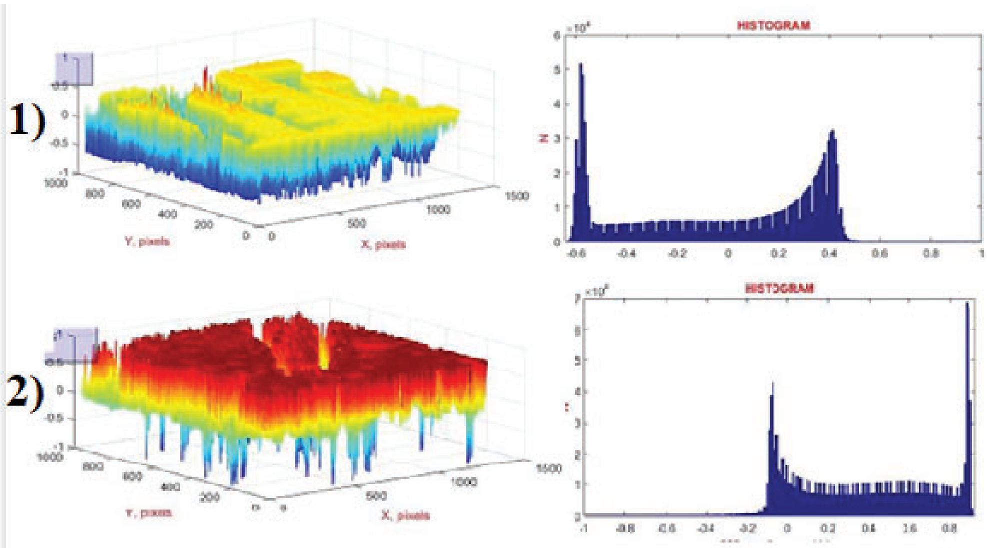


Fig. 1. Distributions of LB values of the crystalline fraction of the VB layers with the TSD of 3 hours (1) and 12 hours (2).

It was established that the coordinate distributions of the LB value of the VB samples of with a greater TSD (12 hours) are characterized by a smaller average value (SM_1=0,68) and a range of dispersion (SM_2=0,34) of random values compared to histograms (SM_1=0,86, SM_2=0,48), that were obtained for the LB of the polycrystalline component of the sample of VB layers with TSD 3 hours (table 1).

Table 1

Temporal dynamics of changes in the value of statistical moments of the 1st-4th orders ($SM_{i=1;2;3;4}$), which characterize the distributions of the LB value of the crystalline fraction of human VB layers with different TSD (T, hour)

$SM_i \times 10^{-2}$	T=1	T=3	T=6	T=12	T=18	T=24
SM_1	0,92±0,043	0,86±0,038	0,81±0,035	0,68±0,031	0,56±0,025	0,41±0,019
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_2	0,53±0,026	0,48±0,024	0,43±0,021	0,34±0,015	0,24±0,011	0,14±0,006
P	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_3	0,71±0,036	0,82±0,041	0,92±0,045	1,13±0,055	1,34±0,059	1,54±0,068
P	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_4	1,21±0,054	1,35±0,058	1,49±0,065	1,76±0,073	2,04±0,092	2,32±0,098
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

We observed that this regularity is associated with destructive changes in the polycrystalline layers of human VB after death. Polarizing manifestations of these changes are accompanied by a decrease in the value of LB, which is determined by the spatial organization of fibrillar collagen networks. With the increase in the duration of the postmortem period, this organization of networks is destroyed, which as a result leads to a decrease in the value of LB.

The statistical analysis of the obtained results showed that the values of the average, dispersion, asymmetry and excess, which characterize the LB maps of the fibrillar collagen meshes of the VB layers of a person with different TSD, change linearly within 24 hours.

The following results were obtained for the accuracy of determination of the TSD by the method of microscopic polarization tomography of LB collagen meshes of layers of VB of a person with different TSD (table 2).

Table 2

Accuracy (±ΔT) of determination of TSD by the method of microscopic polarization tomography of LB of collagen meshes of human VB layers, min

SM_i	T=1	T=3	T=6	T=12	T=18	T=24
SM_1	40	41	41	40	42	43
SM_2	36	38	37	38	38	38
SM_3	24	25	24	25	25	25
SM_4	19	20	20	20	21	21

The analysis of the given results revealed that with the help of this method, the maximum level (highlighted in gray) of accuracy in determining the TSD was achieved within 19-21 minutes. This level of accuracy exceeds the previously known temporal parameters of TSD determination.

Given the fact that postmortem changes in the polycrystalline structure of VB begin with changes in the structure of small-scale protein complexes, the maximum differences between the postmortem states of VB samples with different TSD should be sought on the small scales of changes in the structure of microscopic image maps. Therefore, to improve the effectiveness of the applied methods, a scale-selective wavelet analysis of the coordinate distributions of the obtained parameters of the microscopic images of human VB layers was used.

A comparative analysis of the results of the study of the dependences of the amplitudes of the wavelet coefficients$W_{a, b}$(x, y), characterizing different scales of structural elements of maps of LB collagen meshes of VB layers of deceased people with different TSD, revealed postmortem transformations of the fibrillar structure, the manifestations of which are shown in table 3.

The analysis of the given data revealed an increase to 36 hours in the linear range of the determination of TSD – changes in the value of the set of all statistical moments of the 1st-4th orders, which characterize the maps of wavelet coefficients $C_{a=15, b}$ of the distributions of the LB collagen meshes of the layers of the human VB.

Table 3

Temporal dynamics of changes in the value of statistical moments of the 1st-4th orders, characterizing the distributions of the linear section $C_{15, b}$ of the map of the wavelet coefficients Wa, b of the LB value of the crystalline fraction of VB layers with different TSD (T, hour)

SM_i	T=1	T=6	T=12	T=18	T=24	T=36
SM_1	0,59±0,023	0,45±0,019	0,31±0,014	0,16±0,007	0,013±0,006	0,004±0,002
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_2	0,45±0,021	0,38±0,016	0,32±0,014	0,25±0,011	0,19±0,009	0,054±0,002
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_3	0,31±0,014	0,54±0,024	0,78±0,033	1,01±0,044	1,25±0,054	1,71±0,077
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_4	0,38±0,016	0,72±0,031	1,05±0,044	1,39±0,054	1,72±0,065	2,39±0,108
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

The obtained data illustrate that the values $SM_{1;2;3;4}$, which characterize the distributions of the magnitude of the amplitude of the wavelet coefficients of the maps of the polarization-reproduced LB of the collagen networks of the polycrystalline component of the VB layers of the deceased with different TSD, change linearly within 36 hours. At the same time, temporal changes in the magnitude of asymmetry and excess (SM_3, SM_4) were the most sensitive to postmortem changes in the fibrillar structure of collagen fibers of such samples. Quantitatively, this was manifested in the growth of the angles of inclination of the linear dependences of the statistical moments of higher orders, which characterize the distributions of the amplitude of the wavelet coefficients of the maps of the polarization-reproduced LB of collagen networks, compared to the similar parameters of the maps of the polarization manifestations of the structure of the collagen networks of the humanVB layers with different TSD. Therefore, the sensitivity of the wavelet analysis method increased, and the accuracy of the TSD determination improved by 5 minutes (table 4).

Table 4

Accuracy (±ΔT) of determination of TSD by the method of wavelet analysis of polarization reproduction of the distributions of linear birefringence of the polycrystalline component of the layers of the vitreous body, min

SM_i	T=1	T=6	T=12	T=18	T=24	T=36
SM_1	35	36	36	36	37	37
SM_2	31	32	32	32	33	33
SM_3	18	19	19	20	20	21
SM_4	14	15	15	15	15	16

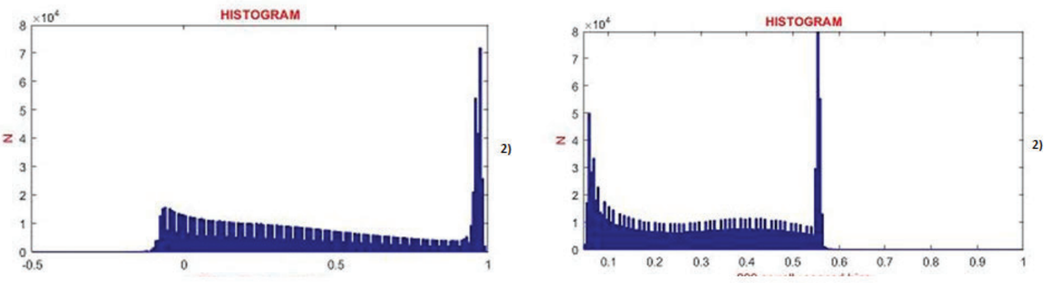
Thus, the effectiveness of the scale-selective wavelet analysis of the LB value distributions of the VB layers at the established TSD has been demonstrated: it provides an increase in the range of sensitivity to 36 hours and an increase in the accuracy of the TSD determination to 14-16 minutes, which was not achieved by any of the existing laser polarization methods.

A new step in the development of methods of optical diagnostics of biological layers was the successful combination of polarimetric and fluorescent methods. Spectral-selective laser fluorescence microscopy is one of the most sensitive methods due to the assessment of concentration parameters of molecules (the greater the number of fluorophores, the higher the fluorescence intensity). The phenomenon of fluorescence is formed as a result of the absorption of optical radiation by various biological compounds with subsequent reradiation into other spectral regions. Due to the differently oriented spatial structure of the fibrils of fluorophores, different directions of oscillations of individual fluorescence bands arise, accordingly, the distribution of azimuths of the polarization of fluorescent radiation is formed, which is of important diagnostic value for the assessment of postmortem changes.

Therefore, the issue of establishing the diagnostic efficiency of spectrally selective laser-induced autofluorescence microscopy of the layers of the VB in the diagnosis of the TSD is relevant.

In our work, two types of interference filters were used for spectral selection of manifestations of different types of fluorophores: λ_1=0,55 μm – amorphous component of the VB; λ_1=0,45 μm – polycrystalline (protein) component of the VB.

Figure 2 shows the distributions of the intensity of laser autofluorescence of the polycrystalline component of the layers of the human VB with the TSD 3 hours (1) and 6 hours (2).



1) 2)

Fig. 2. Distributions of the intensity of the laser spectral-selective autofluorescence (λ_2=0,45 μ) of the polycrystalline component of the human VB layerswith the TSD 3 hours (1) and 6 hours (2).

A «decrease» of laser fluorescence with increasing of the TSD was experimentally demonstrated: coordinate distributions of the value of laser autofluorescence of the polycrystalline component of the VB layers with a larger TSD were characterized by a smaller average value (SM_1=0,52) and the range of dispersion (SM_2=0,25) of random values of the fluorescence intensity compared to the data obtained for the polycrystalline component of the VB layers with a smaller TSD (SM_1=0,78; SM_2=0,42). At the same time, with an increase in the TSD, an increase in the value of SM_3 andexcess SM_4 was noted (table 5).

Table 5

Temporal dynamics of changes in the value of statistical moments of the 1st-4th orders, which characterize the distributions of the intensity of laser spectral-selective autofluorescence of the polycrystalline component of the VB layers with different DNS (T, hour)

SM_i	T=1	T=6	T=12	T=18	T=24	T=36
SM_1	0,83±0,039	0,78±0,033	0,67±0,029	0,52±0,022	0,36±0,014	0,21±0,009
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_2	0,47±0,021	0,42±0,018	0,36±0,015	0,25±0,011	0,15±0,007	0,03±0,002
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_3	0,22±0,009	0,35±0,015	0,48±0,018	0,75±0,031	1,01±0,044	1,27±0,057
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
SM_4	0,11±0,005	0,51±0,022	0,71±0,031	1,11±0,046	1,52±0,069	1,91±0,087
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

The analysis of the obtained data on the time dependence of the postmortem changes in the magnitude of the set of statistical moments of the 1st-4th orders, which characterize the coordinate distributions of the magnitude of the intensity of the fluorescence maps of the polycrystalline fraction of the human VB layers, revealed the highest possible level (highlighted in gray) of accuracy in the determination of the TSD in the range of 15-17 minute for 36 hours (table 6).

Table 6

Accuracy of determination of the TSD by laser spectral-selective fluorescence microscopy of human VB layers, min

SM_i	T=1	T=6	T=12	T=18	T=24	T=36
SM_1	37	37	37	38	38	39
SM_2	34	34	35	35	35	36
SM_3	22	23	23	23	24	24
SM_4	15	15	15	16	16	17

Thus, the analysis of the temporal dynamics of postmortem changes in the magnitude of statistical moments of the 3rd-4th orders, which characterize the distributions of random values of the fluorescence intensity of the polycrystallinecomponent of the VB layers, made it possible to establish a sensitivity range of 36 hours with an accuracy of determining the TSD of 15 minutes.

So, the observed fact of a practically linear change in the magnitude of the statistical moments of the 1st-4th orders, which characterize the distributions of the objective parameters of laser microscopic images of histological sections of biological tissues, became the basis for generalizing the results of the scientists of the school of V. T. Bachynskyi, who initiated a pleiad of studies of biological tissues using polarization techniques. Thus, on this basis, an analytical algorithm was developed to determine and increase the accuracy of establishing of the TSD while maintaining the value of the time intervals.

Conclusions.

1. A comprehensive comparative study of the informativeness of the set of multidimensional polarization and autofluorescence microscopy systems through statistical and wavelet analysis of the temporal dynamics of changes in the optically anisotropic and biochemical structure of the partially depolarizing layers of the VB in the diagnosis of TSD revealed the most informative methods: scale-selective wavelet analysis of coordinate distributions of maps polarization-reproduced LB of collagen networks of the polycrystalline component of the layers of the human VB; spectral-selective laser fluorescence microscopy of the polycrystalline fraction of the layers of the human VB.
2. The paper proved that the use of microscopic polarization tomography and spectral-selective autofluorescence microscopy of images of the VB layers allows determining the TSD within 36 hours with an accuracy of 14-20 minutes.

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МОЖЛИВОСТІ ВСТАНОВЛЕННЯ ДАВНОСТІ НАСТАННЯ СМЕРТІ ЗА ДОПОМОГОЮ МЕТОДІВ БАГАТОМІРНОЇ ПОЛЯРИЗАЦІЙНОЇ Й АВТОФЛУОРЕСЦЕНТНОЇ МІКРОСКОПІЇ СКЛОВИДНОГО ТІЛА ЛЮДИНИ

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Резюме. Враховуючи поточний стан справ у практичній судово-медичній експертизі та значну кількість наукових досліджень з точного визначення посмертного інтервалу, ми приходимо до висновку, що встановлення часу після смерті є ключовою, але поки що недостатньо вирішеною проблемою. Точна оцінка давності настання смерті має вирішальне значення для багатьох розслідувань і залишається однією з найскладніших змінних.

Для проведення точного визначення давності настання смерті важливо застосовувати комплексний підхід, оскільки розв'язання цієї проблеми вимагає урахування всіх аспектів танатогенезу, впливу факторів зовнішнього середовища й інших обставин справи. Це призводить до зростання популярності світових досліджень, що залучають новітні досягнення медицини, хімії, фізики та криміналістики. Використання комплексних досліджень дозволяє наблизитися до встановлення приблизного діапазону давності настання смерті.

Мета дослідження. Розробка комплексу об'єктивних судово-медичних критеріїв для покращення можливостей точного встановлення давності настання смерті на тривалому періоді шляхом застосування багатомірної поляризаційної й автофлуоресцентної мікроскопії скловидного тіла людини.

Матеріали та методи. Об'єктом дослідження були зразки скловидного тіла людей, померлих від серцево-судинної патології, з відомим часом настання смерті.

Використовували наступні методи дослідження: Мюллер-матричне картографування, мікроскопічна поляризаційна томографія, спектрально-селективна лазерно-індукована автофлуоресценція, статистичний і вейвлет-аналізи результатів експериментальних даних.

Результати. Продемонстрована значна ефективність запропонованих методик у точному встановленні давності настання смерті. Зокрема, поляризаційна мікроскопічна томографія з використанням масштабно-селективного вейвлет-аналізу розподілів величини лінійного двопроменезаломлення шарів склистого тіла людини забезпечує діагностичний діапазон визначення давності настання смерті 36 год з точністю до 14-16 хв, що не було досягнуто жодним з існуючих лазерних поляризаційних методів. Водночас поєднання поляризаційної томографії біологічних тканин зі спектрально-селективною лазерною флуоресцентною мікроскопією (одна з найчутливіших методик завдяки оцінці концентраційних параметрів молекул) відкриває нове вікно можливостей у встановленні давності настання смерті та діагностиці різноманітних патологічних станів. Отримані результати підтверджують ефективність даної методики та показують діагностичний діапазон визначення давності настання смерті 36 год з точністю до 15-20 хв.

Висновки. Запропонований у науковому дослідженні інноваційний підхід робить значний внесок у розвиток судово-медичної експертизи, забезпечуючи більш точну та надійну ідентифікацію давності настання смерті, що може бути корисним у кримінальних розслідуваннях та при вирішенні інших судово-медичних завдань.

Ключові слова: судова медицина, скловидне тіло, давність настання смерті, діагностика.

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