POST-MORTEM INTERVAL ESTIMATION USING POLYCRYSTALLINE CEREBROSPINAL FLUID FILMS TWO-DIMENSIONAL CARTOGRAPHY OF MUTUAL LASER POLARIZATION DISTRIBUTION LEVELS

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Abstract: Post-mortem interval estimation is one of the most important issues in forensic practice. Optical diagnostic methods of biological tissue structure assessment are perspective in this area.

The objects of investigation are polycrystalline films of cerebrospinal fluid, taken from 64 corpses with accurately known times of death and 15 healthy volunteers.

The method of two-dimensional stokes-polarimetric mapping of distributions of a complex degree of mutual polarization with spatial-frequency filtration of microscopic images of cerebrospinal fluid films in the time monitoring of post-mortem changes in optical manifestations of polycrystalline networks has been tested in order to estimate the post-mortem interval. The most sensitive post-mortem changes in the optical manifestations of polycrystalline cerebrospinal fluid networks are revealed - statistical moments of the third and fourth orders that characterize the asymmetry and the excess (severity of the peak) of the distributions of values of the complex degree of mutual polarization of large-scale components of cerebrospinal fluid polycrystalline films microscopic images.

An interval of 48 hours with the accuracy of the post-mortem interval estimation in ± 30 minutes were established by the method of two-dimensional mapping of distributions of the values of a complex degree of mutual polarization of large-scale component of cerebrospinal fluid films microscopic images.

Introduction. One of the most important issues of forensic practice is the estimation of the post-mortem interval (PMI) - this issue covers both medical and legal aspects, since the precise definition of this indicator allows more accurate crime investigation (Salam et al., 2012; Arroyo et al., 2005). Physical methods of human biological tissues (BT) research using laser technologies, which include a complex of photometric, polarization and correlation techniques, demonstrated high efficiency of PMI estimation and bodily injuries lifetime. The study of the interrelation between polarization parameters, such as azimuthal distributions and polarization ellipticity, Stokes vector parameters, elements of the Müller matrix, indicators of a complex degree of mutual polarization of images of sections of BT, and the use of statistical analysis, allows us to investigate and interpret the changes in biological objects of research with high accuracy, which was demonstrated in the example of the research of tissues of the parenchymal organs, the skin and the human brain for the diagnosis of PMI (Ushenko et al., 2013; Bachinsky et al., 2000; Ushenko et al., 2011).

Currently the possibilities of objective estimation of PMI by studying posthumous changes in the structure of polarization-heterogeneous microscopic images of human biological fluids are weakly studied (Garazdyuk et al., 2016).

As an object of the study are considered polycrystalline grids of ordered bipolar refractory proteins, forming polycrystalline networks of all basic types of human tissues and liquids. For biological fluids (BF) and environments of the body, a model of supramolecular entities is a liquid crystal analogue, where, as a liquid crystal, which has the form of elongated molecules oriented in one, two or three dimensions, an organized liquid acts with an ordered character of units held by the forces of attraction (Ushenko et al., 2011).

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Aim: to investigate the possibility of diagnosis of PMI and increase the accuracy of its establishment by statistical analysis of the dynamics of posthumous changes in the coordinate distributions of the values of polarization ellipticity of polycrystalline cerebrospinal fluid films (PCF) microscopic images by the method of two-dimensional stokes-polarimetric spatial-frequency mapping of large-scale components of biological layers.

Materials and Methods of Investigation. Objects of investigation were PCF. Two study groups have been formed:

1) the main research group – PCF, taken from 64 corpses with accurately known times of death, who have died from cardiovascular pathology;
2) comparison group – PCF, taken from 15 healthy volunteers.

Cerebrospinal fluid were taken via lumbar puncture (Moghtaderi et al., 2012). PCF were formed in identical conditions by applying a drop to an optically homogeneous glass. The resulting film was dried at room temperature *(t=22°C)*.

The optical circuit and method of experimental study is presented in Figure 1. Figure 1 demonstrates an optical circuit of a laser Stokes polarimeter with spatial-frequency filtering.

Figure 1. Optical circuit of laser Stokes polarimeter with spatial-frequency filtering. Explanation in the text below.
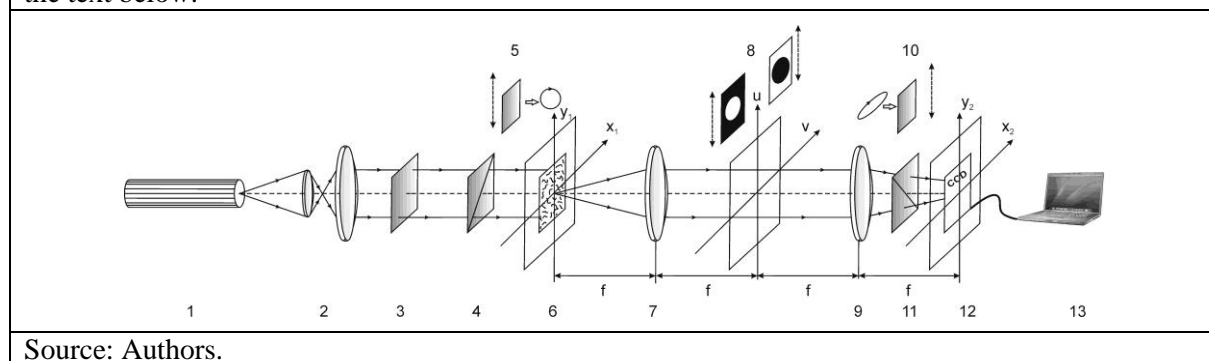
Source: Authors.

The irradiation regime consisted of parallel ($\varnothing = 10^4$ µm bunch of He-Ne semiconductor laser (λ_1=632,8 nm W = 5 mW). The polarization irradiator consists of quarter-wave plate 3, 5 and polarizator 4. Image 6 PCF samples using polarization object glass Nikon CFI Achromat P, the focal length - 30mm, aperture - 0.1, increase - 4x). In the posterior focal plane of the micro-objective 7 space-frequency filter-aperture 8 was placed (low frequency or high frequency). The polarizing micro-lens 9 (Nikon CFI Achromat P, the focal length - 30mm, aperture - 0.1, increase - 4x) was set at the focal length from the frequency plane of the lens 7, and implemented the inverse Fourier transform of the spatially-frequency filtered field of laser radiation (Ushenko et al, 1999).

The coordinate distribution of the intensity of such a field was recorded in the plane of the light-sensitive CCD camera 12 (The Imaging Source DMK 41AU02.AS, monochrome 1/2" CCD, Sony ICX205AL (progressive scan); resolution – 1280x960; photosensitive plane size –7600x6200 nm; sensitivity - 0.05 lx; dynamic range - 8 bit), which was located at a focal distance from the polarization micro-lens 9.

Consequently, the main information objects for the totality of optic-physical methods of diagnostics of the PMI in our work are the set of measured coordinate distributions of the polarization parameters of the spatially-frequency filtered polarization maps of the polycrystalline layer of the liquor.

As the main analytical tool for estimating the distributions of azimuth polarization values of various components of microscopic images of PCF, the statistical moments of the first (Z_1), second (Z_2), third (Z_3) and fourth (Z_4) orders, which were calculated according to the algorithms given by Ushenko et al. (1999), etc. were used.

The method of experimental measurements of coordinate distributions of a module /V/ of complex degree of mutual polarization is indicated in the source (Garazdyuk, 2016)

Two-dimensional mapping of distributions of the degree of mutual polarization of microscopic images of PCF with large-scale spatial-frequency filtration.

Figures 2 and 3 demonstrate results of the study of the time dynamics of degenerative changes in the large-scale component of birefringence-refracting polycrystalline protein networks under conditions of spatial-frequency filtration of coordinate distributions of complex degree of mutual polarization.

Figure 2. The coordinate structure (left side) and the distribution histogram (right side) of the random values of the complex degree of mutual polarization of the microscopic image of the large-scale component of the PCF. Post-mortem interval – 1 hour.
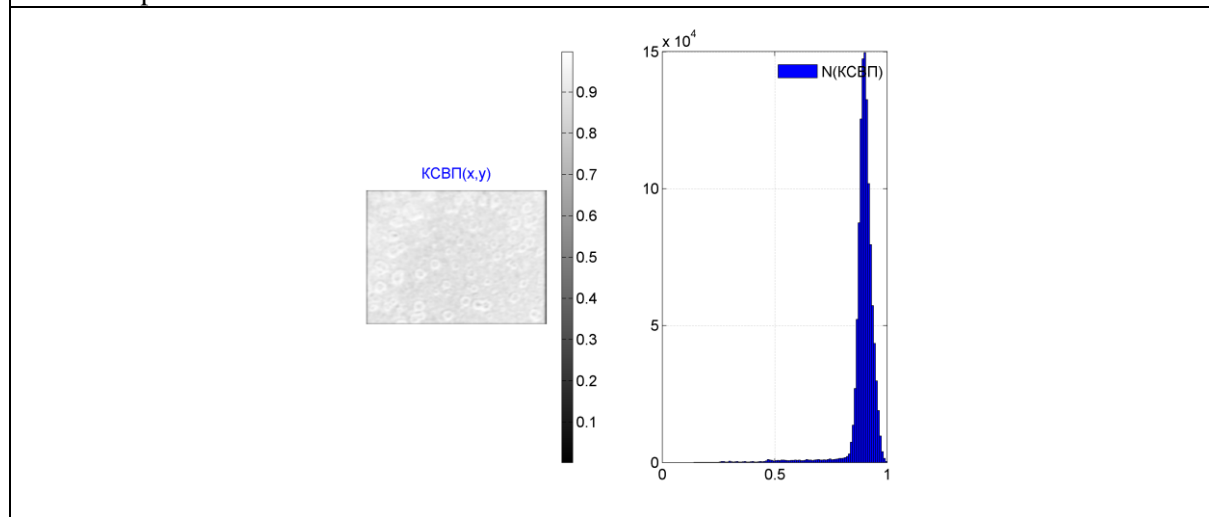
Source: Authors.

Figure 3. The coordinate structure (left side) and the distribution histogram (right side) of the random values of the complex degree of mutual polarization of the microscopic image of the large-scale component of the PCF. Post-mortem interval – 12 hours.
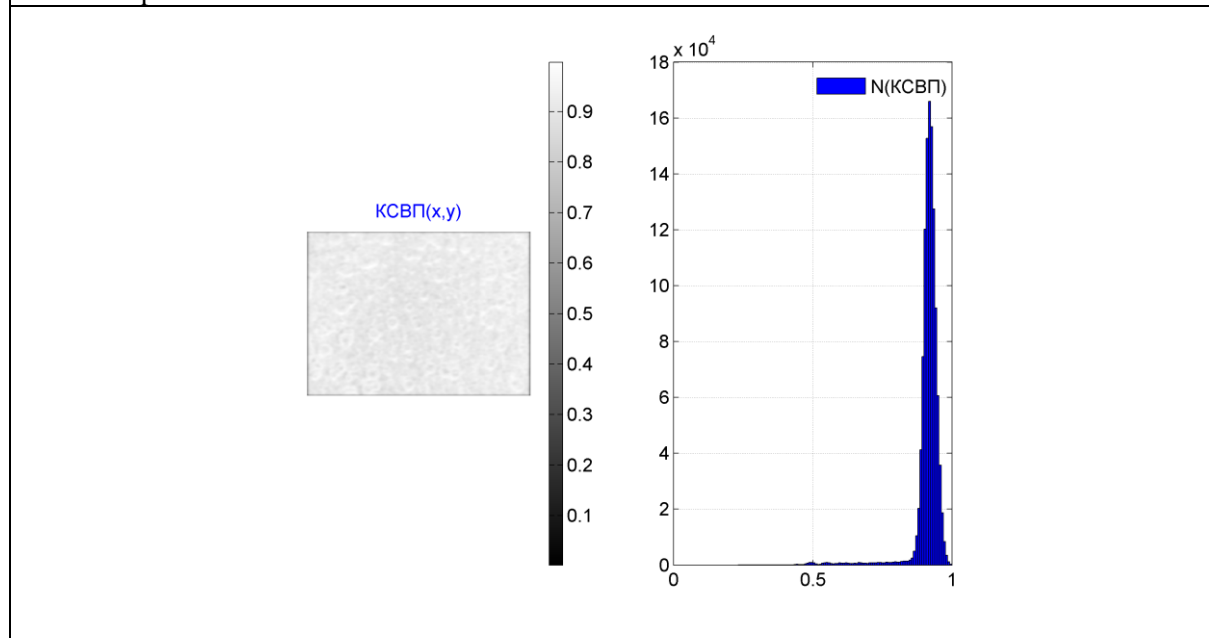
Source: Authors.

From the analysis of two-dimensional polarization-correlation mapping of distributions of values of the complex degree of mutual polarization obtained in the arrangement of a stokes-polarimeter with spatially-frequency filtration of polarization-heterogeneous microscopic images of PCF, it is evident that the degenerative changes of spatially oriented molecular networks at different times of observation after death occur quite slowly (Fig. 2 and Fig. 3, left sides). Quantitatively, this fact illustrates minor changes in the histograms of the distribution of random values of the complex degree

of mutual polarization of a set of points (pixels) of digital microscopic images of large-scale molecular complexes of the PCF (Fig. 2 and Fig. 3, right sides).

Objectively, the process of increasing the interval of determination of the time since death characterizes the comparison of the histograms of the distribution of random values of the complex degree of mutual polarization of large-scale components of microscopic images of PCF. Apparently, the range and dispersion of the random variables of the complex degree of mutual polarization do not significantly decrease (Fig. 2, Fig. 3, right parts) within 12 hours after death.

Such a rate of degenerative changes in the polycrystalline structure can be attributed to the fact that the magnitude of the complex degree of mutual polarization is due to the structure of closely spaced protein complexes. Significant degenerative changes for such structures of the PCF occur at later stages after death. First of all, the degradation of optical anisotropy of PCF is manifested in the destruction of macromolecular protein complexes that have a high level of spatial orientation and, accordingly, a significant birefringence refraction, in medium and low molecular structures with a lower level of optical anisotropy.

Quantitatively, this phenomenon within the statistical approach to the analysis of polarization correlation maps reveals a slight decrease in the mean and dispersion that characterizes the distributions of random values of the complex degree of mutual polarization of the set of points of microscopic images of PCF with an increase in the observation time after death. The statistical moments of higher orders (asymmetry and excess) of such polarization-correlation distributions of the values of a complex degree of mutual polarization are significantly increasing. Therefore, in the process of time monitoring of posthumous changes in polarization-correlation maps of high-molecular protein components of PCF, the most dynamically changing statistical moments of the 3rd and 4th orders, which characterize the asymmetry and the excess of the distribution of random values of the complex degree of mutual polarization of the set of digital points microscopic images.

Table 1 presents the results of calculations of the magnitude of statistical moments of the 3rd-4th order, which characterize the coordinate distributions of values of the complex degree of mutual polarization of the large-scale component of microscopic images of albumin polycrystalline networks, fibrin of PCF for 40 hours after death.
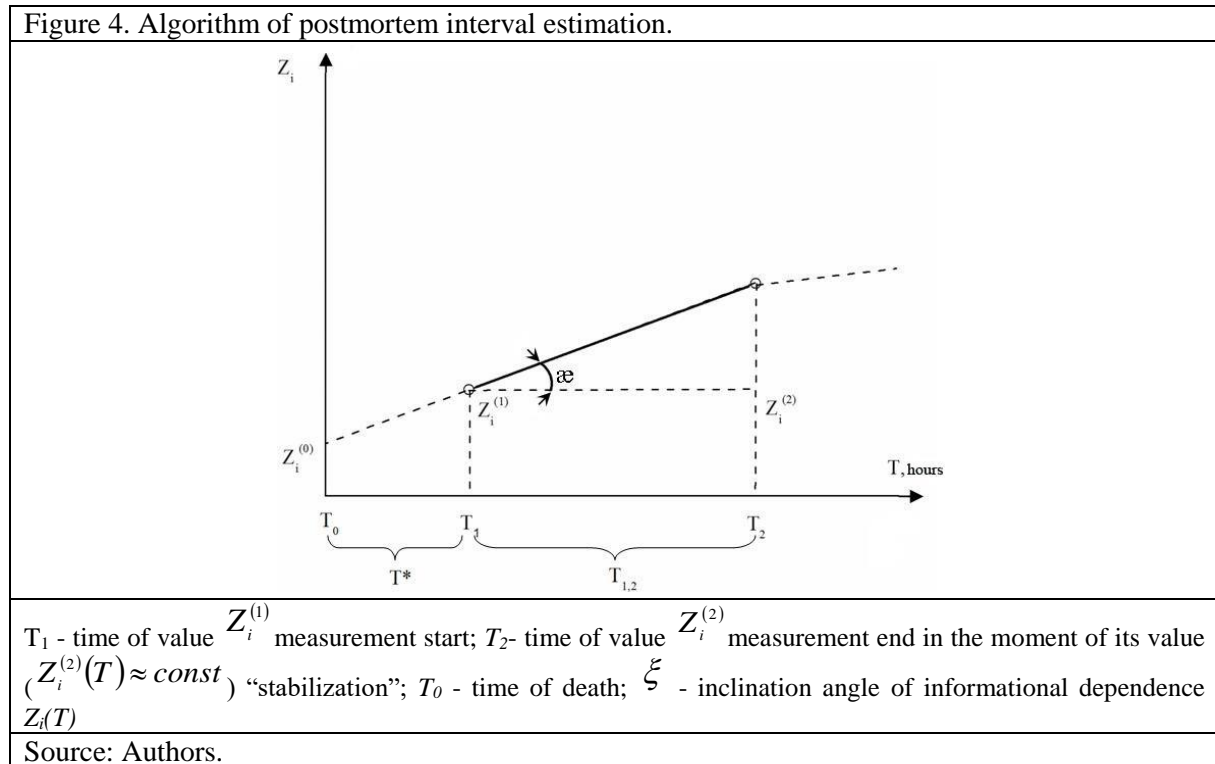
Table 1: Time dependencies of the magnitude of the statistical moments of the 3rd-4th order, which characterize the distributions of the values of the complex degree of mutual polarization of the set of points of the microscopic image of the large-scale component of the PCF of human body						
T, hour	1	8	16	24	32	40
Z_3	0,86± 0,065	1,03± 0,083	1,2± 0,096	1,37± 1,11	1,54± 1,12	1,71± 0,14
P	P<0,001	P<0,001	P<0,001	P<0,001	P<0,001	P<0,001
Z_4	0,83± 0,061	1,02± 0,082	1,18± 0,094	1,34± 0,11	1,51± 0,12	1,68± 0,14
P	P<0,001	P<0,001	P<0,001	P<0,001	P<0,001	P<0,001
T – postmortem interval, hours; Z_3 – statistical moments of the 3rd order; Z_4 –– statistical moments of the 4th order; P – statistical confidence						
Source: Authors.						

An analysis of the time dynamics of the change in the statistical structure of the distributions of the values of the complex degree of mutual polarization of microscopic images of large-scale protein polycrystalline networks of the liquor films revealed:

- The range of changes in the values of the statistical moment of the 3rd order, which characterizes the asymmetry of the distribution of the values of the complex degree of mutual polarization, is 1,89 times during the 40 hours of PMI;
- The range of changes in the values of the statistical moment of the 4th order, which characterizes the excess of the distribution of values of the complex degree of mutual polarization, is 2,1 times during the 40 hours of PMI.

Analytical algorithm to determine postmortem interval

From the analysis of the model scheme, shown in Fig. 4, we can get the following relationships to determine the PMI

Figure 4. Algorithm of postmortem interval estimation.

T_1 - time of value $Z_i^{(1)}$ measurement start; T_2- time of value $Z_i^{(2)}$ measurement end in the moment of its value ($Z_i^{(2)}(T) \approx const$) "stabilization"; T_0 - time of death; ξ - inclination angle of informational dependence $Z_i(T)$

Source: Authors.

$$tg\xi = \frac{Z_i^{(1)} - Z_i^{(1)}}{T_2 - T_1} = \frac{\Delta Z_i^{(1,2)}}{\Delta T_{12}} \ . \tag{1}$$

Using equation (2), we obtain the equation to determine time since death:

$$T^* = Z_i^{(0)} \frac{\Delta T_{12}}{\Delta Z_i^{(1,2)}} \ . \tag{2}$$

Here: $Z_i^{(0)}$ - objective value, defined by a fence CSF collecting in vivo in healthy volunteers (Table 3).

Table 3: Statistical moments of the 3rd and 4th order, which characterize PCF microscopic images maps of the complex degree of mutual polarization

Z_i^0	W*	W**	
Z_3^0	0,79±0,064	0,31±0,023	
P	P<0,001	P<0,001	
Z_4^0	0,64±0,053	1,43±0,12	
P	P<0,001	P<0,001	
P – statistical confidence			
Source: Authors			

The following intervals and the accuracy of postmortem interval have been established: estimation interval – 48 hours, estimation accuracy – 60 min.

Conclusions

The statistical moments of the third and fourth orders that characterize the asymmetry and the excess (severity of the peak) of the cerebrospinal fluid polycrystalline films microscopic images values of

large-scale components distributions of the complex degree of mutual polarization are the most sensitive optical manifestations of post-mortem polycrystalline cerebrospinal fluid network changes.

The proposed method allows estimation of the post-mortem interval within 48 hours after death with an accuracy of ± 30 minutes.

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