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A. POPOV[1], V. KOZHEMYAKO[2], S. TUZHANSKY[2], A. KRAIEVSKA[2]

ELECTRONIC SPECLE-PATERN INTERFEROMETRY COMPARATIVE ANALYSIS AND APPLICATION IN NON-INVASIVE BIOMEDICAL RESEARCHES

[1]*Research Institute of Physics, Odessa I. I. Mechnikov National University*
65082, Paster st., 27, Odessa, Ukraine

[2]*Vinnitsa National Technical University*
21021, Khmelnitske Shose st., 95, Vinnitsa, Ukraine
Cell: +380632746910, E-mail: a.kraevska@gmail.comt

Abstract. Electronic speckle-pattern interferometry is considered in this paper as a non-invasive method which allows to avoid disadvantages of holographic interferometry and provides precise measurements in vivo of morphological and dynamical characteristics of biological objects. This allows numerous diseases diagnosing and predicting. Research of human saliva morphological characteristics are presented in this paper

Анотація. В цій статті розглядається спекл-інтерферометрія як неінвазивний метод, який дозволяє усунути недоліки голографічної інтерферометрії і забезпечити точне вимірювання морфологічних і динамічних характеристик живих біологічних об'єктів. Це надає можливість діагностування і прогнозування багатьох хвороб. Також в цій статті надається дослідження морфологічних характеристик людської слини.

Аннотация. В этой статье рассматривается спекл-интерферометрия как неинвазивный метод, который позволяет избежать недостатков голографической интерферометрии и обеспечить точное измерение морфологических и динамических характеристик живых биологических объектов. Это предоставляет возможность диагностики и прогнозирования многих болезней. Также в этой статье предоставлено исследование морфологических характеристик человеческой слюны.
Keywords: Electronic Speckle-Pattern Interferometry (ESPI), specklegram, speckle, interferometry, holography.

INTRODUCTION

Studying of morphological and dynamical characteristics of biological objects in vivo for cardiovascular and lymphatic systems diseases diagnosing, eye diseases diagnosing, internal organs monitoring is actual problem of biology and medicine. Nowadays interferometric methods for precise measurements are widespread in many areas of science and technology. In particular, noninvasive monitoring and diagnostics methods based on holographic interferometry are under investigation and elaboration. Such methods are characterized by extremely high sensitivity, and they are much easier in usage than classical interferometric methods. Registered deformation range is from tenths of micron to hundreds of microns. Holographic method variety, informativity and clearness provide an effective and fast tool for optical noninvasive control. However, holographic interferometry method usage is associated with a hologram recording. That's why it is very toilful and requires highly skilled staff. Also expensive equipment is another disadvantage of holographic method [1].

Method based on specklegram processing using computer correlation is more widespread recently. This method is known as ESPI — electron speckle-pattern interferometry or as phase-modulated speckle interferometry. This method allows to combine the advantages of two approaches — holographic interferometry and speckle interferometry and allows to eliminate their disadvantages. ESPI method is widely used in medicine and in other fields. Some approaches of ESPI method are elaborated and patented by employees of Physics Institute in Odessa National University [1].

HOLOGRAPHIC INTERFEROMETRY

Surrounding objects are visible for human eye because they become the light sources — they either reflect or refract the incident light waves. Reflected waves overlap, interfere and form the wave surface known also as wave front which extends from the object in space. This wave front has full information about object — amplitude, wavelength and phase. Our eyes react to the wave front, which extends from the object and do not

react to the object itself. Therefore, if we could capture the wave front somehow and restore it in convenient for us time the observer would not be able to distinguish object illusion image from the real object in this case.

Wave amplitude may be recorded by white-black photography, wave amplitude and wavelength may be recorded by color photography. But there were no phase detectors, which can record information about the object size. This issue was decided by Dennis Gabor, who proposed a new 3D image recording method in 1947. This method was called holography, what in Greek means a complete recording [1].

Holography is based on the fundamental laws of wave optics — interference and diffraction laws. Two waves are needed to be overlapped in order to realize wave registration and renewal. These are wave which is reflected by object and wave coherent to it.

Laser beam is split into two separate beams of light in order to record a hologram of a complex object. One beam illuminates the object, which scatters light into the recording medium. According to diffraction theory, each point in the object acts as a point source of light. So the recording medium can be considered as illuminated by a set of light point sources located at varying distances from the medium.

The second (reference) beam illuminates the recording medium directly. Each point source wave interferes with the reference beam, giving its own sinusoidal zone plate increasing in the recording medium. The resulting pattern is the sum of all these 'zone plates' which are combined to produce a random (speckle) pattern as in the photograph below (pic. 1).

When the hologram is illuminated by the original reference beam, each of the individual zone plates reconstructs the object wave which produced this plate. And these individual wavefronts reconstruct the whole object beam. The viewer perceives a wavefront that is identical to the wavefront scattered from the object into the recording medium. And it appears to the viewer that the object is still in place even if it has been removed. This image is known as a «virtual» image, as it is generated even though the object is no longer there. The object and reference beams are mathematically determined by the following formula:

$$A_n = A_{01}\cos\left(\omega t - \frac{2\pi}{\lambda} r_1 + \phi_1\right), \tag{1}$$

$$A_o = A_{02}\cos\left(\omega t - \frac{2\pi}{\lambda} r_2 + \phi_2\right), \tag{1'}$$

where A_{01}, A_{02}, r_1, r_2, φ_1, φ_2 — are the amplitudes, distance between light source to recording medium and start phase accordingly [1].

Overlapping these two harmonic waves gives another resulting harmonic wave. Resulting harmonic wave is determined:

$$A_o = A_0\cos(\omega t + \phi). \tag{2}$$

Amplitude and start phase of this wave are described by the following formula:

$$A_0^2 = A_{01}^2 + A_{02}^2 + 2A_{01}A_{02}\cos\left(2\pi\frac{r_1 - r_2}{\lambda} + (\phi_2 - \phi_1)\right). \tag{3}$$

As intensity is proportional to amplitude squared, resulting intensity in the recording medium is determined:

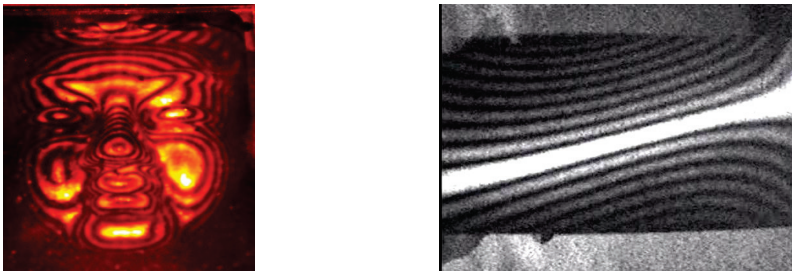
$$I = I_1 + I_2 + 2\sqrt{I_1 I_2}\cos\left(2\pi\frac{r_1 - r_2}{\lambda} + (\phi_2 - \phi_1)\right), \tag{4}$$

$$\Delta\phi = \left(2\pi\frac{r_1 - r_2}{\lambda} + (\phi_2 - \phi_1)\right), \tag{5}$$

where $\Delta\phi$ — phase difference [1].

So phase difference does not depend on time. It depends on recording medium location. Such waves are called standing waves and they are called interference images in optics. So all information about object is presented as intensity distribution in space, which is registered on photoplate. Image renewed from photoplate by illuminating it with reference beam has the same phase as registered object wave. This renewed image is called a hologram.

If two holograms of one object are recorded on one photoplate and phase structures of object beams are different (due to object deformation, laser wavelength change, etc.) the renewed image is covered by interference fringes. Location of these interference fringes describes phase changes. This is the principle of double-exposure holographic interferometry (pic. 1) [1].



a) b)

Pic. 1. Holographic interferograms of stationary object (mask): a) recorded by two-mode laser emission; b) interferogram of plate which is distorted between expositions

Interferograms analyze allows to determinate deformations and shape of objects and other useful parameters. But as it is shown on pic.1 such analyze cannot be full without additional information because using only these data it is impossible to determinate the sign of phase changes.

Electronic Specle-Pattern Interferometry (ESPI) allows to avoid such disadvantages. Also it allows to realize all advantages of holographic interferometry using the principally new base. This method is widely explored by scientists from Odessa National Technical University [2, 3].

PRINCIPALS OF ELECTRONIC SPECLE-PATERN INTERFEROMETRY (ESPI)

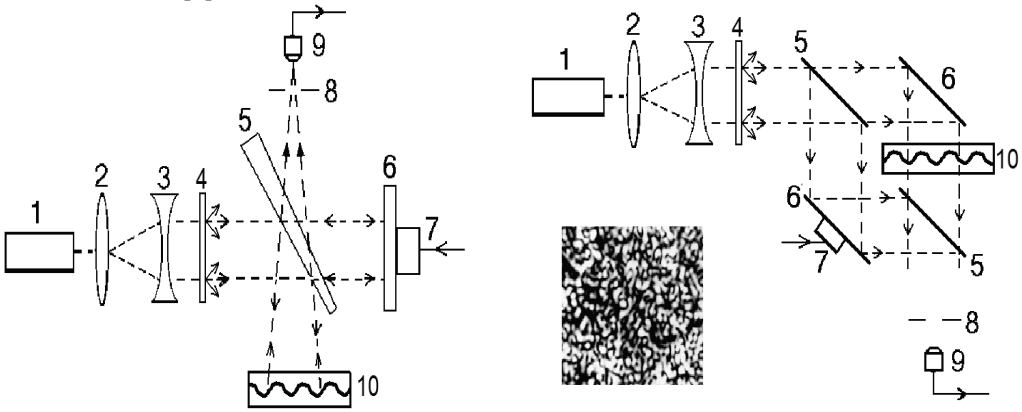
Method ESPI is contiguous to digital (computer) holographic methods and it concerns coherent (laser) emission usage.

Method ESPI is based on object wave phase portrait composing using additional reference phase-modulated wave.

Analysis of phase portrait changes (determination of speckles phase correlation) allows to determinate object deformations, object shape and refractive index variations precisely.

Measurements are based on specklegram set recording (each specklegram has appropriate phase shift which is determined by computer) using camera and their further correlation analyze.

So ESPI method is based on experimental measurement of object beam phase structure. This can be archived for coherent or particularly coherent beams only. That's why actually all ESPI devices are speckle type laser interferometers [3].



a) b) c)

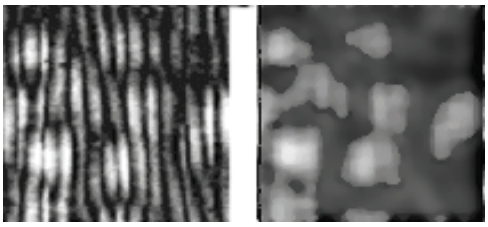
Pic. 2. Speckle-interferometers: a) Michelson interferometer; b) specklegram; c) Mach-Zehnder interferometer

Optical schemes for ESPI method realization are represented on pic. 2. These are speckle-interferometers which are similar to Michelson interferometer for opaque body research (pic. 2a) and Mach-Zehnder interferometer for transparent body and translucent body (pic. 2c) researches. Example of specklegram is represented on the pic. 2b.

The following components are represented on the pic. 2a and 2c: 1 — laser, 2 and 3 — lens, 4 — diffuser, 5 — divider, 6 — mirror, 7 — device to control phase shift (piezoceramics), 8 — diaphragm, 9 — camera, 10 — sample.

Object, reference and summary beams have speckle structure (pic. 2b) in represented ESPI method. This speckle structure is caused by diffuser 4. Speckle size in summary specklegram depends on diaphragm 8, which filters the beam. This makes beam convenient to camera resolution. Specklegram is registered by camera 9.

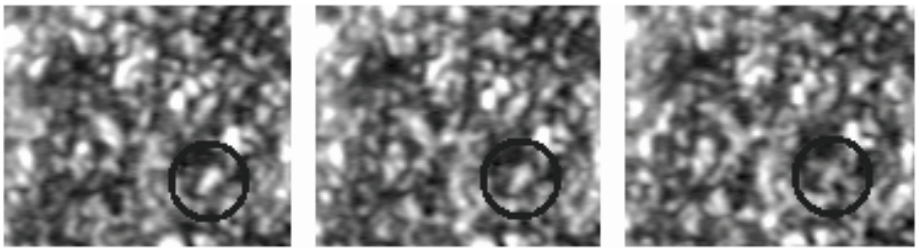
Emission phase is constant within one speckle. Emission phase is randomly changed within all speckles. This is a special feature of speckle structures. Interference fringes are observed if object and reference speckle beams are overlapped in summary beam (specklegram). Spatial frequency of these interference fringes depends on beams ascent angle. Location of interference finger minimums and maximums depends on spackle phase differences which are overlapped in object and reference beams (pic. 3a). Interference fingers location will be shifted according to phase change in one of beams. But interference fingers are not observed in specklegram (pic. 3b) after spatial filtering. Speckles intensity depends on speckles phase difference which are overlaped in object and reference beams [3].



a) b)

Pic. 3. Specklegrams with different diaphragm 8 diameter

Changing reference beam phase (using voltage which is provided to piezoceramic 7) speckle intensity in specklegram is changed (pic. 4). This is equivalent to the transition from the regime of the interference fringes with finite width to the regime of fringes with infinite width.



Pic. 4. Set of 3 specklegrams with reference beam phase shift: 0, π/2 and π accordingly. It is shown one speckle intensity change (in circle)

Intensity distribution in each point $\vec{r}$ of specklegram registration plane is determined by the following formula:

$$I_1(\vec{r}) = I_a(\vec{r}) + I_b(\vec{r}) + 2\sqrt{I_a(\vec{r})I_b(\vec{r})}\cos\Delta\phi(\vec{r}) \qquad (6)$$

where $I_a(\vec{r})$, $I_b(\vec{r})$ and $\Delta\phi(\vec{r})$ — are spatial intensities distribution and phases difference of interfering object and reference speckle-beams accordingly. The resulting intensity distribution $I_1(\vec{r})$ in interference pattern depends on not only spatial distribution $I_a(\vec{r})$ and $I_b(\vec{r})$, it depends on phase differences $\Delta\phi(\vec{r})$ of interfering beams also [1].

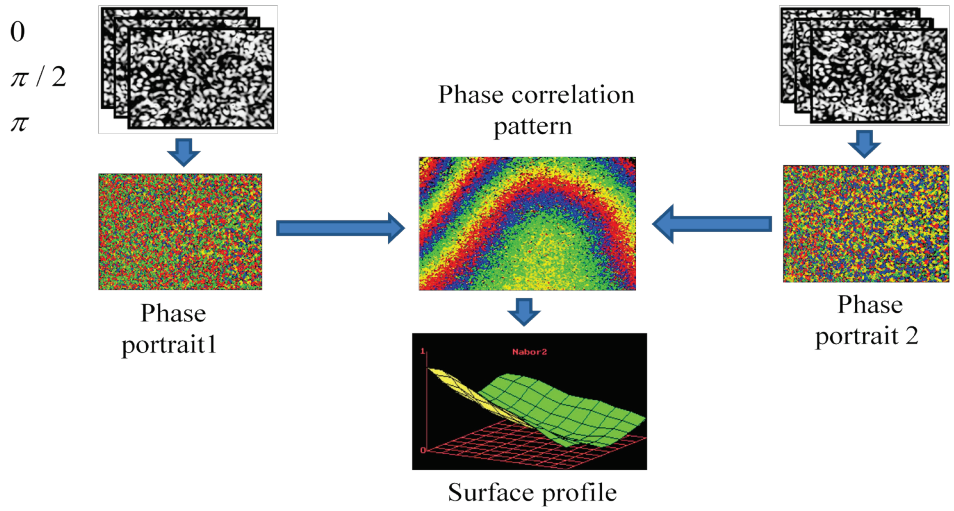
Correlation processing of specklegram set which is recorded with few calibrated phase shifts of object beam allows to get spatial distribution $\Delta\phi(\vec{r})$ and phase portrait of object beam further. 3 phase shifts of object beam are needed as minimum to get phase portrait precisely. Phase shifts: 0, π/2 и π have been used in our case. Phase shifts number may be increased for more precise measurements. Such specklegram set registration (recording) is called measurement cycle and is the same as hologram registration in holographic interferometry [2].

Two measurement cycles are needed to determinate phase difference in object beam, which is formed by two different object states. Calibrated phase portrait of object beam is determined by formula 6 in the first cycle when surface is known to be a plane and if it is not possible sample for comparison is used. Second and next cycles (if there is an interest in phase distribution change dynamic in object wave) — include phase portrait forming of object beam which is reflected from object.

Phase distribution change in object wave leads to additional phase shift appearing $\Delta\Psi(\vec{r})$ between object and reference beams. Intensity distribution in summary beam (specklegram) in registration plane will be changed and will be determined by the following formula:

$$I_2(\vec{r}) = I_a(\vec{r}) + I_b(\vec{r}) + 2\sqrt{I_a(\vec{r})I_b(\vec{r})}\cos[\Delta\phi(\vec{r}) + \Delta\Psi(\vec{r})] \quad (7)$$

Calibration phase portrait comparison with phase portrait which are formed in second and next cycles allows to remodel additional phase shift $\Delta\Psi(\vec{r})$ in each point of registration plane for different time moments precisely. In other words it allows to form phase correlation pattern [3].



Pic. 5. Schema of Electronic Specle-Pattern Interferometry (ESPI)

So ESPI method allows to realize all possibilities of double-exposure holographic interferometry. ESPI method advantage is unambiguity of phase correlation pattern decoding analogical to interfegograms (pic. 5).

ELECTRONIC SPECLE-PATERN INTERFEROMETRY APPLICATION AND TOOLS

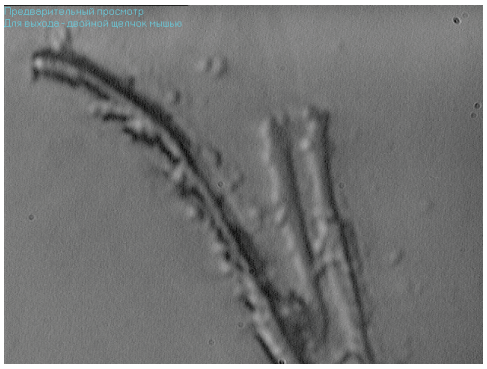
ESPI method is realized using special equipment developed by scientists of Odessa National Technical University [2, 3].

This equipment costs much chipper than holographic microscope. But it allows to realize features of holographic microscope:

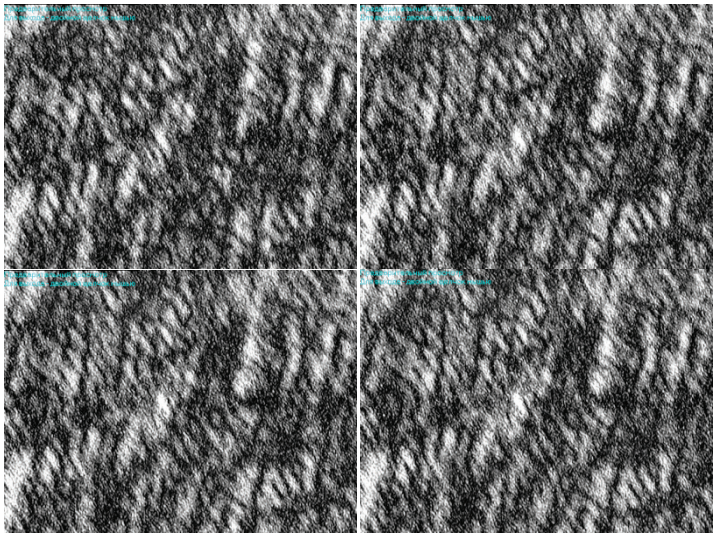
— To get object phase portrait;
— Nano expansion;
— High level of performance;
— Allows to determinate optical characteristics of cells and organelles (refractive index, anisotropy, optical density, etc);
— There is possibility to investigate nano-dynamic in vivo;
— There is no need in special modifications of alive samples;
— There is no need in device calibration [3].

Human saliva has been investigated as a sample for ESPI method. Human saliva view via optical microscope is presented on the pic. 6.

Specklegrams of human saliva are presented on the pic. 7. These specklegrams have been got using ESPI method.

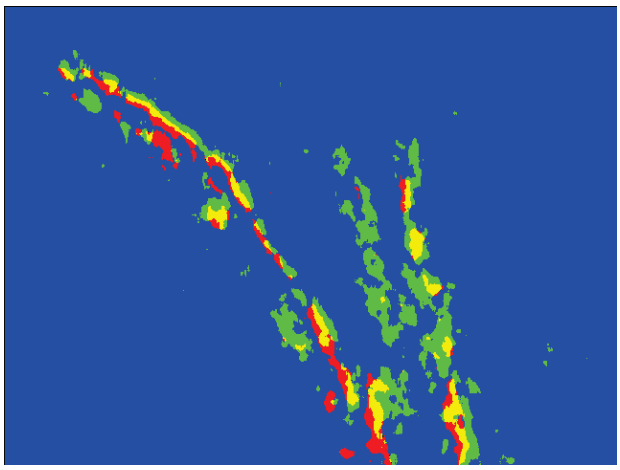


Pic. 6. Human saliva view via optical microscope



Pic. 7. Human saliva speclegrams

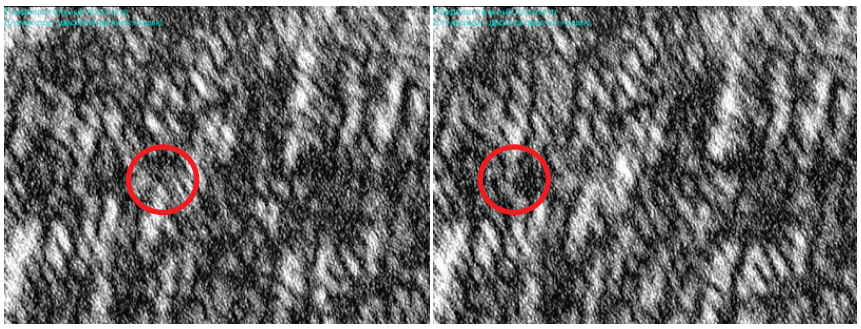
Human saliva phase portrait is presented after correlation analyze on the pic. 8. Phase values: 0 — blue, $\pi/2$ — green, π — yellow, $3\pi/4$ — red.

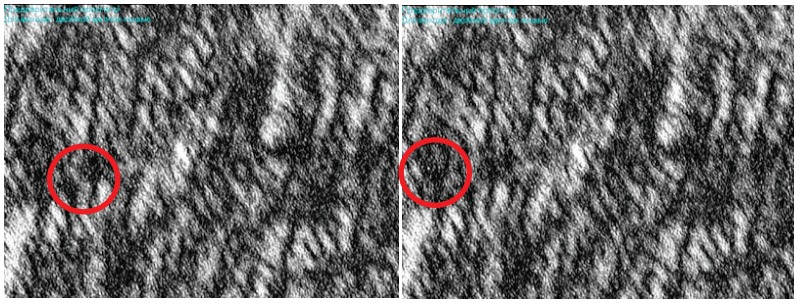


Pic. 8. Human saliva phase portrait

In order to improve speclegrams processing special software was developed by scientists from Vinnitsa National Technical University. This software allows to upload greyscale bmp images and process them.

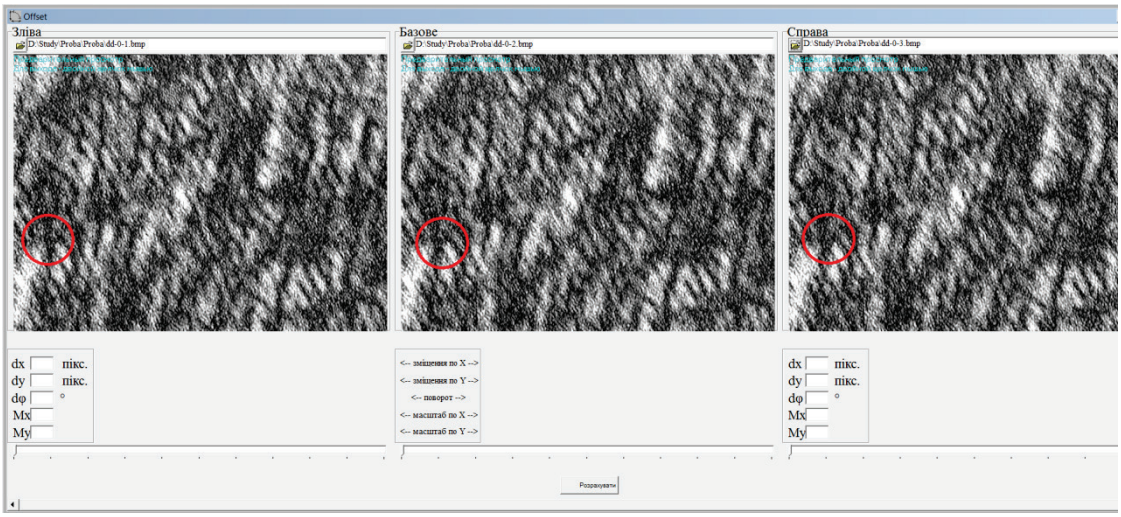
For example let's proceed with speclegrams from pic.7. Red circles highlight object which we want to investigate.





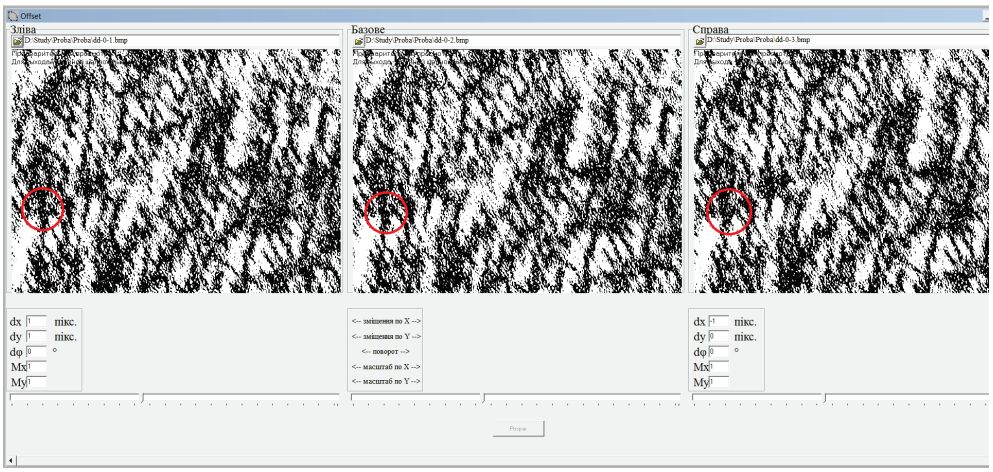
Pic. 9. Human saliva speclegrams with highlighted areas for investigation

Uploaded speclegrams are presented on the pic. 10.



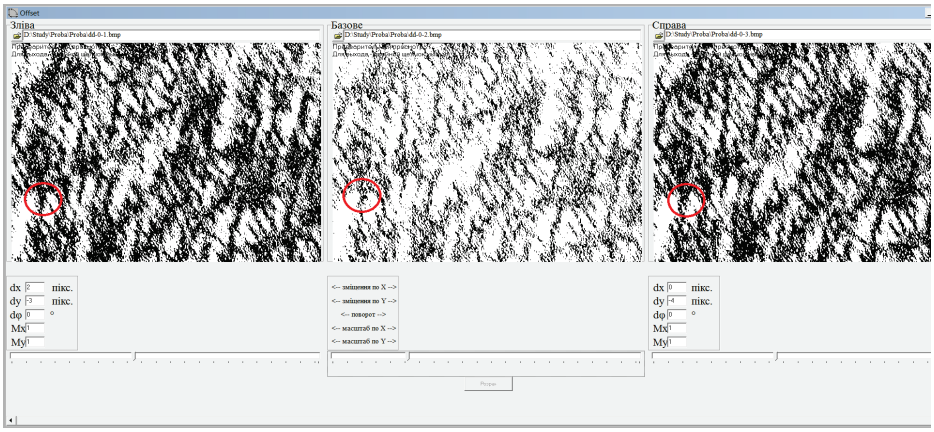
Pic. 10. Human saliva speclegrams after uploading to the software

After images are uploaded we can click «Розрахувати» button. This will determine deviation by X-axis and by Y-axis, rotation, scale by X-axis and by Y-axis. Example of processed speclegrams is displayed on the pic. 11.



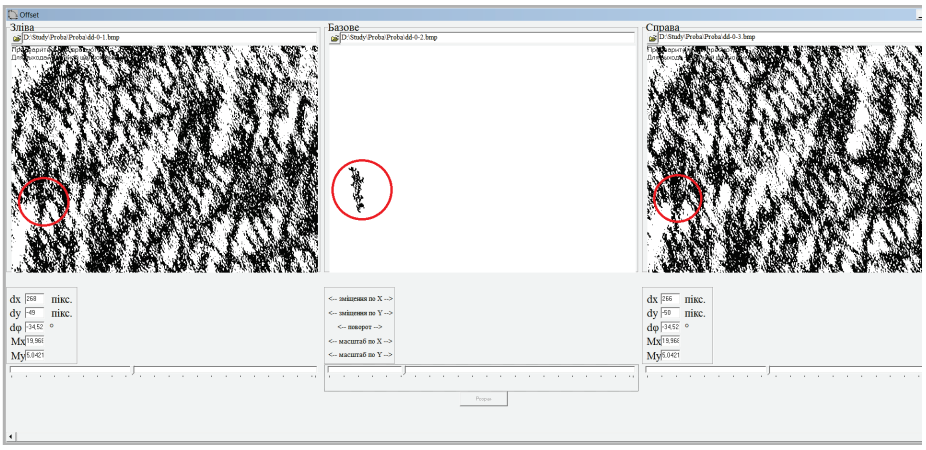
Pic. 11. Human saliva speclegrams after processing

Also we can increase or decrease the image brightness in order to differentiate object from the background. Example is displayed on the pic. 12.



Pic. 12. Human saliva speclegrams after object differentiation

Object can be allocated for further investigations. Example is displayed on the pic. 13.



Pic. 13. Human saliva speclegrams after object allocation

This software helps to analyze speclegrams and precisely allocate the object for further investigations and 3d image modeling using ESPI method.

CONCLUSION

ESPI is widely used in ophthalmology to detect cornea forms and elastic characteristics of eye tissues. ESPI is an effective method to detect level of capillary blood flow (microcirculation disturbances assessing), to diagnose some diseases on early stage. ESPI method is an effective instrument for internal organs and tissues diagnosing with optical fibers usage. Analyzing performance of lymphatic system ESPI is used for flow monitoring in lymphatic microvessels. Also ESPI is widely used in surgery for stress-strain state investigation of skull and jaw bone tissue under pressure, for cartilage deformation investigation.

Presented ESPI device is much chipper and it is available analog of holographic microscopes. And it has the same technical features.

Advantage of ESPI device is compatibility with almost all optical microscopes and their complexes.

Measurement sensitivities may vary from 0.01 to tens of microns in depth depending on method realization.

Extension is determined by microscope and camera magnifications (to 1 micron).

Measurement time is varied from tenth of second to few seconds.

Speclegrams received using ESPI device and optical microscope can be processed by developed software. This software allows to analyze speclegrams, precisely differentiate and allocate object for further investigations.

So Electronic Specle-Pattern Interferometry application allows to get quantitative data about morphological and dynamical characteristics of biological objects, allows to investigate them in vivo and under the influence of various factors solving the actual questions of biomedical diagnostics.

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ПОПОВ А. Ю. — к. ф.-м. н., завідувач науково-дослідної лабораторії прикладної фізики і комп'ютерних технологій у Одеському національному університеті імені І. І. Мечникова, м. Одеса, Україна.

КОЖЕМ'ЯКО В. П. — д. т. н., проф., зав. кафедри лазерної та оптикоелектронної техніки у Вінницькому національному технічному університеті, м. Вінниця, Україна.

ТУЖАНСЬКИЙ С. Є. — к. т. н., доцент кафедри лазерної та оптикоелектронної техніки у Вінницькому національному технічному університеті, м. Вінниця, Україна.

КРАЄВСЬКА А. В. — аспірантка кафедри лазерної та оптикоелектронної техніки у Вінницькому національному технічному університеті, м. Вінниця, Україна.