Special training laboratory on optical biophysics. Education-research setups for postgraduate students

Ivan V. Fedosov, Alexey N. Bashkatov, Elina A. Genina, Georgy V. Simonenko, Dmitry A. Zimnykov, and Valery V. Tuchin[1]

Saratov State University, Saratov, RU 410026

ABSTRACT

The set of educational-research practical works for postgraduate students of the special training laboratory on optical biophysics is described. Presented materials were also discussed on SPIE 7th International Conference on Education and Training in Optics and Phoptonics 2001 (ETOP), 26-30 November, 2001.

Keywords: optical biophysics, spectroscopy, scattering media, speckles, Doppler system, color imaging, skin, training laboratory.

1. INTRODUCTION

This paper presents the set of practical works of the training laboratory on optical biophysics[1-5] for postgraduate students. This set of practical works consists of the following setups:

1. Laser Doppler velocimeter
2. Speckle-interferometric instrument for monitoring of capillary bio-flow
3. Two-wavelength laser scanning microphotometer
4. Spatial RGB analyzer of biological objects
5. Spatial-resolved microspectrophotometer for tissue optical properties and geometry studies: CCD tester

The requirements to a learning person: background in light scattering, tissue optics and spectroscopy.[6-8]

2. LASER DOPPLER VELOCIMETER

This practical work will enable students: to understand principles of optical heterodyning and digital signal processing, to get an overview of principles and schemes of laser instruments such as laser Doppler anemometers (LDA)

Basic kit: laser Doppler velocimeter; rotating screen with motor; set of transparent tubes and scattering fluid.

Optical scheme of experimental setup is presented in Fig.1. A typical Doppler frequency shift power spectrum obtained with rotating disk can be found in Fig. 2.

Examples of practical tasks available with the basic kit:

1. Investigation of physical principles of LDA using rotating scattering screen.
2. Investigation of flow velocity distribution in the tube for Newtonian and non-Newtonian (whole blood) liquids.
3. Development of digital signal processing and analysis for flow dynamics monitoring.

[1] Department of Physics, Saratov State University, 155, Moskovskaya str., Saratov, 410026, Russia; tel/office: +7 (8452) 51-46-93, fax: +7 (8452) 24-04-46; e-mail: tuchin@sgu.ru

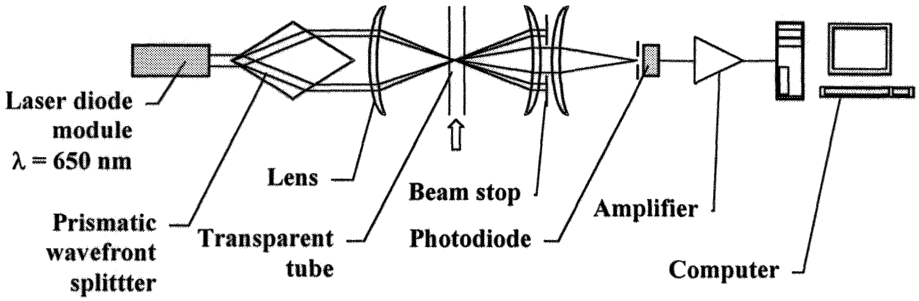

Fig. 1: Optical scheme of laser Doppler velocimeter

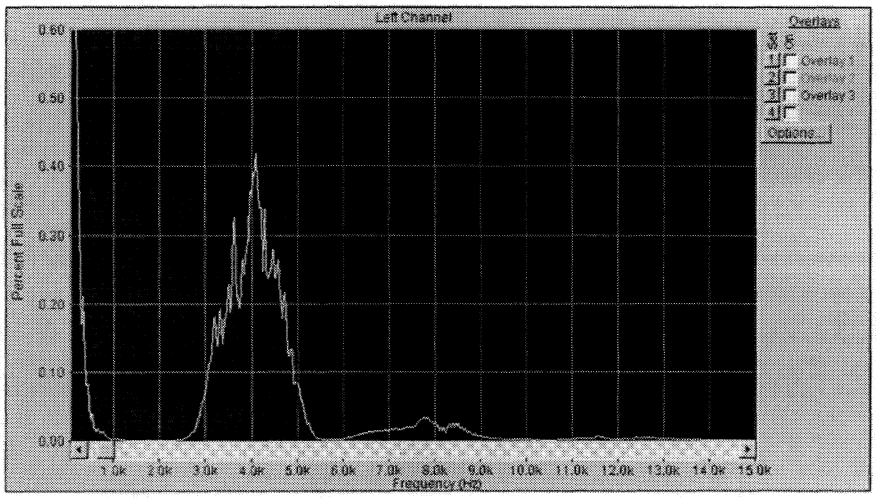

Fig.2: Doppler frequency shift power spectrum obtained with rotating disk.

3. SPECKLE-INTERFEROMETRIC INSTRUMENT FOR MONITORING OF CAPILLARY BIO-FLOW

This practical work will enable students: to get an overview of dynamic speckles properties; to identify the time correlation-spectral and space correlation approaches in speckle dynamics analysis; to understand the basic principles of homodyne photodetection and speckle-interferometry.

Experimental setup is presented in Fig. 3. It allows for bio-flow velocity and direction monitoring and investigation of speckle field statistical properties.

Two plots characterizing space-time correlation of dynamic speckle field: the space-time correlation function and calibration curve for translation velocity of speckles – are presented in Figs. 4 and 5.

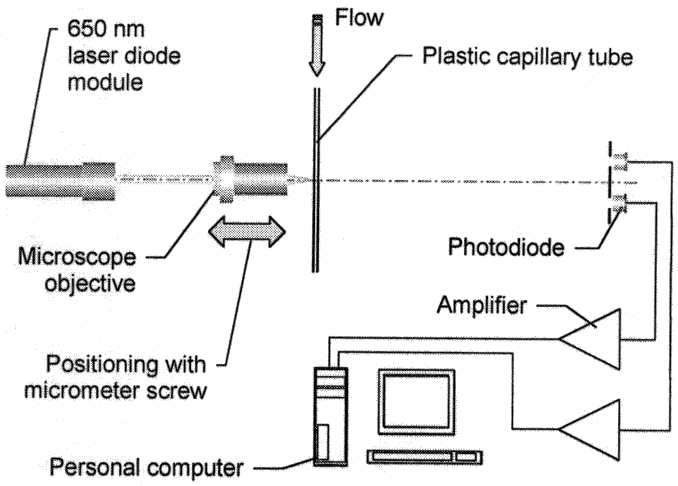

Fig.3: Speckle-interferometric instrument for monitoring of capillary bio-flow.

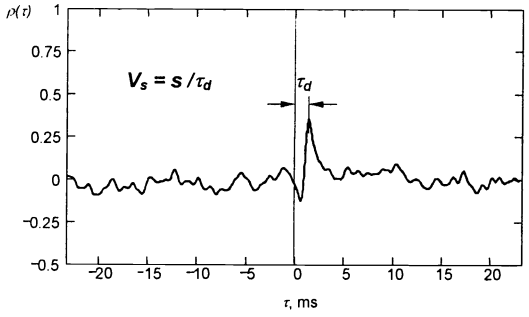

Fig. 4: Dynamic speckle field space-time correlation. Correlation function estimate.

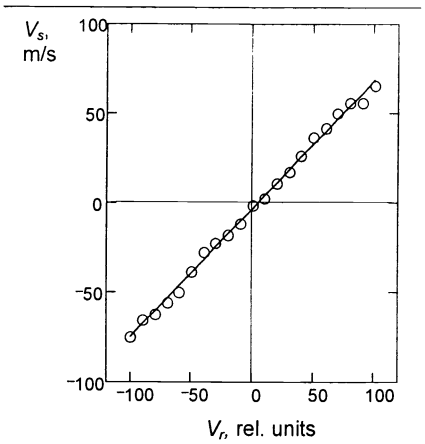

Fig.5: Plot of speckle translation velocity

Examples of tasks for students:

1. Investigation of dynamic speckle field time-dependent intensity fluctuations in a fixed point.
2. Investigation of dynamic speckle field space-time correlation.
3. Monitoring of non-stationary capillary flow.

4. TWO-WAVELENGTH LASER SCANNING MICROPHOTOMETER

This practical work will enable students: to get an overview of tissue optical properties; to understand principles of photometry; to analyze spectral images of tissue samples.

Optical scheme of two-wavelength laser scanning microphotometer is presented in Fig. 6. Single and wo-wavelength images in Fig. 7 illustrate the diagnostic possibilities of the instrument.

Examples of tasks for students: investigation of pigment absorption at different wavelengths and concentrations; imaging of tissue sample at two wavelengths; programming of automated sample scanning.

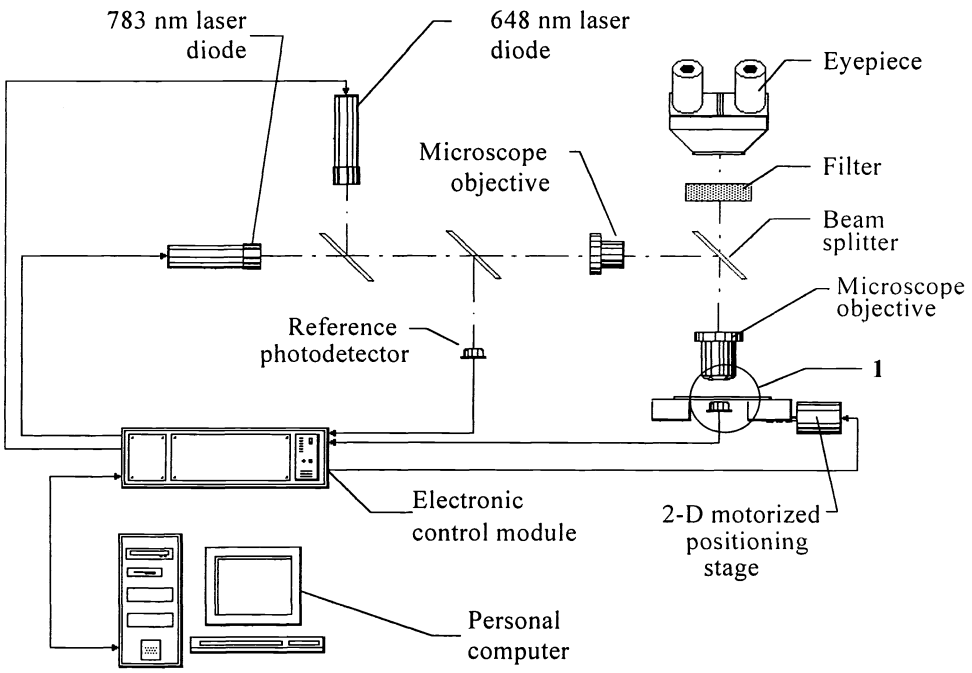

Fig. 6: Optical scheme of two-wavelength laser scanning microphotometer.

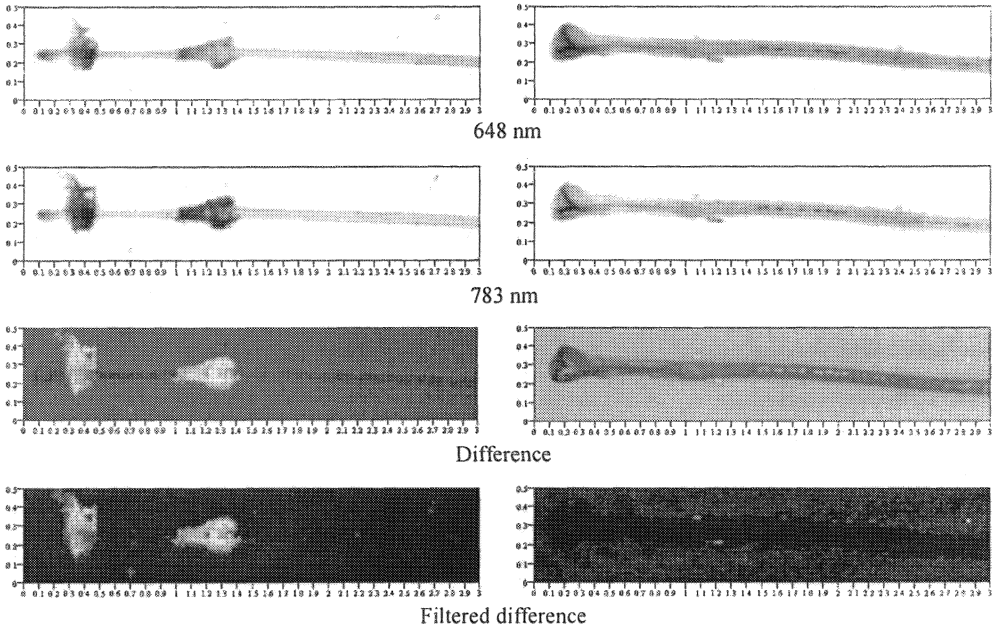

Fig. 7: Images of the epilated human hairs at single and two wavelengths (difference and filtered difference). I*n vivo* stained by Indocyanine green (left images) and natural (right images) hairs.

5. SPATIAL RGB ANALYZER OF BIOLOGICAL OBJECTS

This practical work enables students: to obtain knowledge of principle of digital analysis of color image of biological objects; to study technique for measurement of transmittance and reflectance of biological objects using their digital images; to study inverse Monte Carlo method for estimation of tissue optical properties; to estimate optical properties of human hair by inverse Monte Carlo method and spatial digital image analysis.

Digital imaging is a method whereby images are represented by a series of numbers. Each number usually represents a measure of energy reflected from a tiny elemental portion of the structure that is being imaged. In two-dimensional imaging, this tiny picture element is called a *pixel*, is usually rectangular, and is displayed as a single dot in digital image. Color resolution (pixel depth) refers to the number of bits of information that are used to represent either the number of shades of gray or number of colors that each pixel can represent. Eight bits of information can represent 2^8=256 shades of gray or 256 different colors. In 24-bit true color images, 8-bits (256 shades) for each of three primary colors (usually red, green, and blue) have been using.

To evaluate the clinical morphology of different skin lesions (pigmentation, psoriasis, erythema, etc.), measurement of hair growth, wound healing, and burn management digital imaging methods have been applying. Digital imaging techniques combined with inverse Monte Carlo method can be also used for estimation of optical properties of human hair shafts.

To obtain optical properties of the human hairs the following steps have to be done: (1) to record images of the hair in both reflectance and transmittance modes using experimental setup (Fig. 8); (2) to process obtained images with the developed software allowing for getting of selected red, green, and blue components of the image; (3) to determine reflectance and transmittance of the hair for each color components; (4) to calculate the optical properties of a hair shaft (absorption and reduced scattering coefficients) using experimental data for reflectance and transmittance and inverse Monte Carlo method.

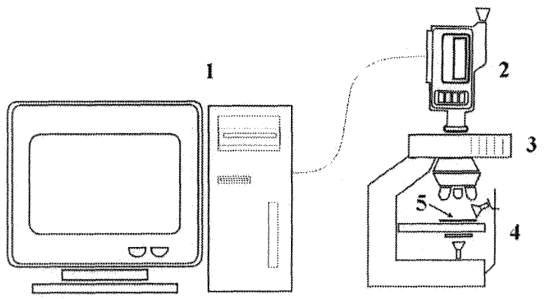

Fig. 8: Video-microscopic system for spatial digital color analysis of biological objects: 1 – PC, 2 – CCD-camera, 3 – light microscope, 4 – light sources for transmittance or reflectance measurements, 5 – biological object.

The color imaging system is composed of a video-microscope (SVHS Sony CCD-TR617E, PAL, Japan (2) and light microscope (3)) interfaced with a personal computer (1). The specimen (plane plate with attached biological object) (5) is illuminated by white light (halogen lamps (4) provide illumination for recording of transmittance or reflectance images. In dependence on mode of the illumination the specimen plate presents either transparent glass plate (transmittance mode) or black & white test-object to provide the similar conditions of registration of images (reflectance mode).

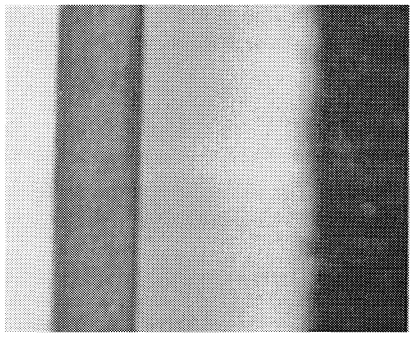

Fig. 9: Image of the human hair shaft (left) on the background of the black & white test-object, recorded in the reflectance mode (x 200).

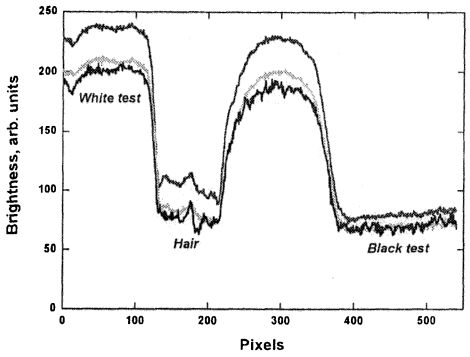

Fig. 10: The typical averaged scans of the hair shaft image for color components corresponding to three spectral ranges (red, green, and blue).

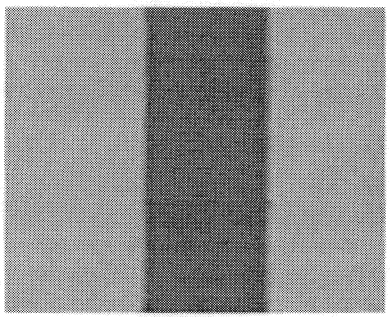

Fig. 11: Image of the human hair shaft recorded in the transmission mode (x 200).

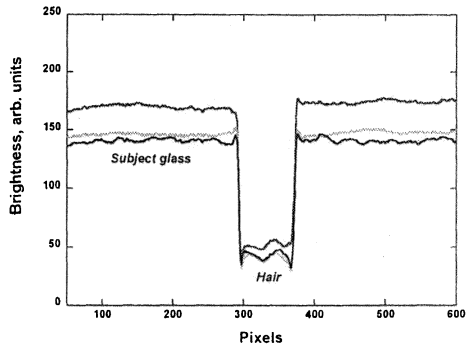

Fig. 12: The typical averaged scans of the hair shaft image for color components corresponding to three spectral ranges (red, green, and blue).

Using measured transmittance and reflectance and inverse Monte Carlo method, absorption coefficient μ_a and reduced scattering coefficient μ'_s of the hair shaft at three spectral ranges can be estimated (see Table 1).

Table 1. Optical properties of different hair shafts obtained by inverse Monte Carlo method. All values were averaged over 30 samples.

Hair color	Red component		Green component		Blue component	
	μ_a, 1/см	μ'_s, 1/см	μ_a, 1/см	μ'_s, 1/см	μ_a, 1/см	μ'_s, 1/см
Black	28.5 ± 6.3	37.5 ± 18.8	38.6 ± 7.7	53.4 ± 35.6	68.3 ± 8.99	91.3 ± 91.3
Brown	14.5 ± 3.8	40.6 ± 17.4	19.71 ± 5.7	55.8 ± 29.01	34.82 ± 8.9	90.5 ± 48.3
Light brown	1.16 ± 0.064	105.9 ± 45.1	1.58 ± 0.061	142.3 ± 71.3	2.79 ± 0.13	223.2 ± 167.3
Blond	0.45 ± 0.074	161.1 ± 40.3	0.78 ± 0.12	172.2 ± 42.5	1.93 ± 0.28	229.1 ± 91.6
Grey	0.35 ± 0.054	233.6 ± 63.6	0.6 ± 0.1	268.3 ± 83.01	1.48 ± 0.2	331.4 ± 118.2

The differences between optical properties of various types of hair shafts are well seen. These differences are directly connected with hair shaft structure. High scattering observed for gray hairs is explained by the presence of air-bubbles within the hair shaft. The different content of melanin granules within various shafts (black, brown, etc) causes difference in absorption properties. Melanin granules having higher refractive index than surrounding medium (keratin) also give input in light scattering.

Student tasks:

1. Estimation of transmittance and reflectance of different types of human hair shafts using their digital images;

2. Estimation of absorption and reduced scattering coefficients of the human hair shafts;
3. Analysis of spectral dependence of the absorption and reduced scattering coefficients;
4. Explanation of the differences between optical properties of different types of hair shafts types on the basis of their morphology.

6. SPATIAL-RESOLVED MICROSPECTROPHOTOMETER FOR TISSUE OPTICAL PROPERTIES AND GEOMETRY STUDIES: CCD TESTER

The optical scheme of spatial-resolved microspectrophotometer for tissue optical properties and geometry studies: CCD tester is presented in Fig. 1. Object under study is placed in the object plane of the imaging lens (microscope objective with magnification equal to 8 and numerical aperture equal to 0.20) and is illuminated by a fiber-optic illuminator (cross-section diameter is equal to 6 mm) assembled with interference filters (bandwidth centered at 600, 700 or 800 nm). Prism is used to change the optical axis direction. Image of the part of the object is formed on the photosensitive area of the black & white spectral CCD camera (Electrim 1000). Camera operation is supported by the special software developed by camera producer – Electrim Inc. This software allows one to save images of the object under study in 8-bit bitmap format. Saved image can be processed by the special MathCad (MathSoft Inc., USA) program allows one to find 2D distributions of the object transmittance for selected wavelength (600, 700 or 800 nm) and to measure its geometrical parameters. For example, for given number of pixels in rows and columns of CCD chip (192×165 for non-interlace mode) the number of pixels in the transverse direction of the hair shaft image is approximately equal to 35 (for 50-μm hair diameter). To measure the hair diameter preliminary calibration by using precision 50-μm grid on the glass substrate is applied.

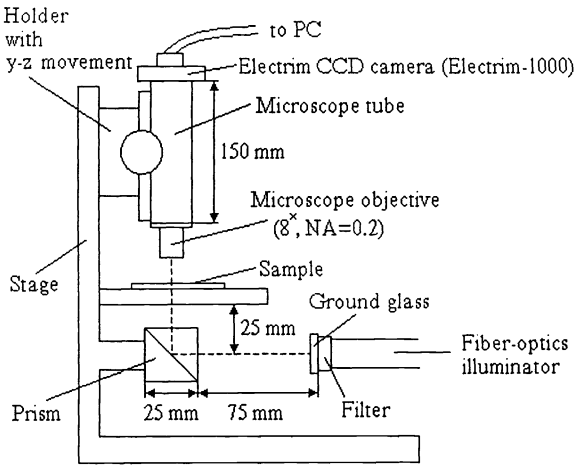

Fig.13: Optical scheme of spatial-resolved microspectrophotometer for tissue optical properties and geometry studies: CCD tester

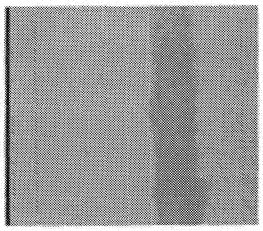

Fig. 14: The CCD image of the hair shaft.

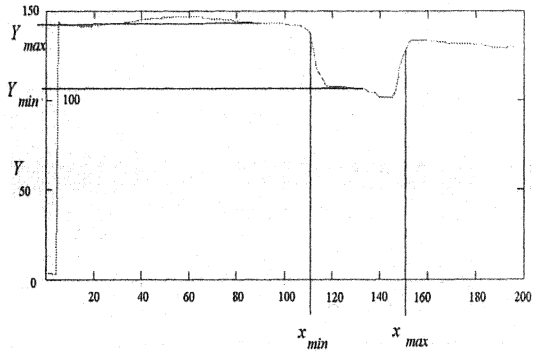

Fig. 15: The transverse distribution of hair shaft transmittance.

To define transmittance of an object the ratio of intensity of transmitted light in the area of the object's image I_h to intensity of light transmitted out of this image I_r - reference, can be taken. In addition the background signal defined by the dark current of CCD elements I_b should be subtracted from both hair shaft and reference signals. Thus transmittance is defined as

$$T = \frac{I_h - I_b}{I_r - I_b} .$$

The optical density D, absorbance Abs, and attenuation (turbidity) μ_t at three wavelengths 600, 700, and 800 nm can be calculated

$$D = logT,$$
$$Abs = lnT,$$
$$\mu_t = Abs/d,$$

where d is the object thickness or diameter.

Table 2. Measured and calculated transmission (T), absorbance (Abs) and attenuation coefficient (μ_t) for hair shafts and their mean square deviations (SD) averaged for 27 samples. Attenuation coefficient (μ_t) was calculated for diameter d=50 μm.

Parameter	CCD, λ=624 nm	CCD, λ=700 nm	CCD, λ=800 nm
T, %	55.9±8.8	60.6±6.5	71.6±10.6
Abs	0.61±0.16	0.50±0.10	0.33±0.08
μ_t, cm^{-1}	122±42	100±37	66±25

Student tasks:

1. To measure geometric parameters of the human hair shafts.
2. To measure transmission and calculate absorbance and attenuation coefficient of the human hair shafts.
3. To measure erythrocytes size changes at glucose action.

7. CONCLUSION

These practical works enable students: to understand principles of optical heterodyning and digital signal processing; to get an overview of principles and schemes of laser instruments, such as laser Doppler anemometers (LDA), and of dynamic speckles properties; to identify the time correlation-spectral and space correlation approaches in speckle dynamics analysis; to understand the basic principles of homodyne photodetection and speckle-interferometry; to get an overview of tissue optical properties; to understand principles of photometry; to analyze spectral images of tissue samples; to obtain knowledge of principles of digital analysis of color image of biological objects; to study technique for measurement of transmittance and reflectance of biological objects using their digital images; to study inverse Monte Carlo method for estimation of tissue optical properties; to estimate optical properties of a tissue by inverse Monte Carlo method and spatial digital image analysis.

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