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Teaching of optical diffraction methods in Biomedicine to undergraduates specializing in Optics

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ABSTRACT

The report presents a circulation plan of the course on Diffraction Methods in Biomedicine. When teaching the course, the special attention is drawn to an analysis of strongly focused Gaussian beams diffraction in random media. Original materials included in lectures provide the most complete study of the question. The diffraction fundamentals are taught in accordance with Rytov, Kravtsov and Tatarsky's book under the title of "Introduction in Statistical Radiophysics". Delivering lectures is combined with practical undergraduate training in speckle optics problems and seminars. The seminars are organized as scientific conferences. Along with theoretical course the laboratory training is foreseen. In particular, the paper describes two set-ups designed for investigations of human pulse waves, cardiovascular vibrations and for measurements of white rats lymph and blood flows in vivo.

Keywords : speckles, scattering, biomedicine.

1. INTRODUCTION

At the present the Biomedical Optics is one of the fast developing directions of modern optics. Industry and science indicate a high demand in the professionals specializing in this field. Nevertheless, this speciality is presented in the Universities of Russia poorly enough. In Saratov State University the MSc program in Biomedical Optics started ten years ago. One of the courses taught since 1991 is "Diffraction Methods in Biomedicine". This course includes 17 lectures delivered for undergraduates (fourth-year students).

2. STRUCTURE OF CIRCULATION PLAN OF THE COURSE

In general, the proposed course of lectures on strongly focused coherent beams scattering is dedicated to the teaching of basic physics of methods of biomedicine and design of diagnostic methods utilizing diffraction phenomenon. Mostly these are the methods characterized by high locality of measurements. The structure of the course is following.

Chapter I: Fundamentals of random diffraction. Speckles formation

Helmholtz equation. Kirchhoff method of solution of diffraction problems. Green functions of I and II orders. Diffraction of plane wave by the screen with a hole. Diffraction of coherent light on random and periodic motionless phase screen. Speckles. Correlation functions of the random fields, formed in the far zone of diffraction. Developed and partially-developed speckle-fields. Diffraction of wide coherent beams in random media. The first and the second orders statistics of phase and intensity fluctuations in speckles. Dynamics of scattering of the plane wave by a small particle.

Chapter II: Speckles dynamics

Diffraction of strongly focused Gaussian beams from moving rough surface and in scattering flow. Formation of dynamic statistically inhomogeneous speckles in different zones of diffraction. Correlation functions of amplitude, phase and intensity fluctuations of dynamic statistically inhomogeneous speckles. The first and the second orders statistics of intensity fluctuations in the far zone of diffraction. Dependencies of statistical characteristics of dynamic statistically inhomogeneous speckles on properties of scattering media and on the conditions of speckles observation. Dynamics of statistically inhomogeneous speckles with non-Gaussian statistics.

Chapter III : Speckled speckles dynamics

Diffraction of focused speckles from moving phase screen. The first and the second orders statistics of doubly scattered speckles. Dynamics of doubly scattered speckles. Dependence of statistical properties of dynamic speckled speckles on the statistics of moving random phase screen.

Chapter IV : Interference of dynamic statistically inhomogeneous speckle-fields

The peculiarities of manifestation of the Doppler effect at focused Gaussian beam diffraction in weakly scattering moving media. The differences in these manifestations in the cases of focused Gaussian beam diffraction by moving random surface and by the scattering flow. Interference of two dynamic statistically inhomogeneous speckles. The first and the second orders statistics of intensity fluctuations of interfering dynamic statistically inhomogeneous speckle-fields. Manifestation of the interference effects with focused Gaussian beams diffraction from moving multilayered random medium.

Chapter Y: Biomedical diagnostics using focused Gaussian beams diffraction

Brief overview of speckle-interferometrical methods for biomedicine diagnostics. Bio-speckles. Speckle-interferometer for measurements of human cardiovibrations and pulse waves. Alterations of the structure of space-time projections of interferometer output signal at different human heart disorders. Methods of biovibration analysis based on the usage of focused Gaussian beam diffraction from the surfaces both of human skin and thin rubber membranes contacting with the investigated surface. Focused Gaussian beam diffraction in narrow blood capillaries in vivo. Measurements of blood and lymph flows parameters in native capillaries. Doppler and speckle-interferometrical methods of flowmetry using focused Gaussian beam scattering. Principles of diffraction cytometry.

Chapter YI: Focused Gaussian beam diffraction applied for the measurements of biospecimens structure

Brief overview of laser diffraction methods of surfaces diagnostics. Laser profilometry, based on the focused Gaussian beam diffraction. Scanning laser profilometer. Its amplitude, phase and amplitude-frequency characteristics in linear mode of work. Higher harmonic components of laser profilometer output signal in non-linear mode. Multifrequency regime of work of laser profilometer. Analysis of random surfaces with usage of laser profilometer. Metrological characteristics of laser profilometer. Analysis of the structure of upper layers of human epidermis used for skin diseases diagnostics. Investigations of the process of the growing of bacteria colonies with the usage of scanning laser profilometer.

3. SOME METHODOLOGICAL PECULIARITIES OF TEACHING OF THE COURSE

In general the process of the course teaching may be divided into three stages. The first stage concerns to the studying of the fundamentals of light diffraction by objects having random structure. The learning of this part of course is carrying out in the form of students conferences: each student of the class has to make a presentation dedicated to some subject from the curriculum plan and then his report has to be discussed by the class. The materials containing in the Refs. 1-3 are recommended for the study. At the mentioned stage the Professor teaching the class makes only a critical comments when the presentation had made and discussed. In the opinion of the authors of the course such style of teaching allows to the students to get an experience of making a scientific public presentations.

The second part of the course (chapters II- IV) dedicated to the studying of speckles dynamics with a small number of scatterers is professed in the traditional way, i.e. Professor gives a lectures to the class. The third part of the course is dedicated to the biomedical diagnostics utilizing the diffraction methods of measurements. At this stage the delivering of the lecture course is accompanied by the carrying out of some practical works.

4. TWO PRACTICAL WORKS

4.1. Methods of speckle-optics for biovibration detection

The first practical work is dedicated to cardiovibrations measurements. The purpose of this work to give the students the understanding of the mechanism of measurement signal formation. This work contains three exercises.

Exercise a. Vibration analysis with the use of differential and Michelson speckle-interferometers

The output signal of the interferometer is expressed⁴ as $U = A \sin(AL \sin H(t) + \varphi)$, where A and φ are the random values (φ - uniformly distributed one). The characteristics of normalized output signal depends not only on the form of signal $H(t)$, but also on the amplitude of signal difference AL (in wavelength) of surface oscillations in two points of light perfusion and the initial phase φ . For differential speckle - interferometer the vibration amplitude $AL \ll 1$, distortion of

speckle -interferometer input signal $H(t)$, caused by non-linear effects, is small, that is why the differences between the space-time projections of function $H(t)$ and output signal of the interferometer are not considerable.

Using the differential speckle interferometer the pulse waves monitoring is carried out. Optical signal is registered at the point of space where speckle-fields are matched. The vibration analysis, fulfilled with non-different scheme using, is characterized by a non-small parameter AL , which this time is the total scattering surface vibration amplitude. So, when the speckle-interferometer operates in this regime, the non-linear effects take place. The input signal of interferometer $H(t)$ is essentially distorted.

Exercise b. Method of shear skin surface vibration analysis based on focused Gaussian beams diffraction on the surface

The normal skin vibration is accompanied, as a rule, by small shear oscillations; both types of oscillation have the similar spectra. At the focused Gaussian beam diffraction the statistically inhomogeneous partially developed speckles are formed. At the focused Gaussian beam diffraction the shear shifts of scattering surfaces result in dynamics of the partially developed speckles in the far zone of the diffraction. When the surface oscillates periodically, the scattered field intensity also has a periodical component. The intensity fluctuations $I(t)$ are related to the surface motion law $X_s(t)$ by some functional dependence, such as $I(t) = F(X_s(t))$, where F is a nonlinear random operator. The operator form depends on the focused Gaussian beam parameters and the conditions of the speckle-field observation, as well as on the particular random realization of the skin rough surface. Students are to register the realizations of the measuring system output signal.

Exercise c. Analysis of the skin surface vibration using the focused statistically inhomogeneous speckles diffraction

The scheme of biovibrometer⁵ utilized in this exercise is given in Fig.1. In this scheme the focused Gaussian beam passes through the optical fiber 1 and then partially developed speckle-field, formed in this fiber, is focused by the microobjective onto a thin rubber membrane covered with a reflecting coating. The reflected field passed again through the microobjective is received by the regular fiber 2 and then led to photomultiplier, which sends electric signal to recorder's amplifier. The non-linear distortion of the measuring vibration signal does not exceed 70 %. The sample pulsegram registered by the students with the help of the described device is presented in Fig.2.

4.2. Laser speckle-anemometer for biological fluids diagnostics

The second practical work is dedicated to the blood or lymph flow analysis in native capillaries. The optical scheme of the setup used for experimental investigation is shown in Fig. 3. Laser beam is focused into the spot with the small diameter ($D=2.9$ mcm) in the investigated vessel. Blood flow modulates the strongly focused Gaussian beam in the waist plane. This leads to the speckles dynamics in Fraunhofer zone of diffraction. Optical microscope supplied by the TV-camera allows to observe the lymph flow in a microvessel visually. Considered experimental set-up and the theory of the measurement have been presented in Ref.6 which is recommended to the students as a methodological material. Experiments are performed on white noninbred rats, narcotized by intra muscular injection of Nembutal. Mesentery obtained through an incision in the anterior abdominal wall is placed on the thermostabilized microscope stage. Carrying out this practical work, students have to register the spectra of intensity fluctuations of the speckles scattered from native vessel. In Fig.4 the typical spectra obtained for strongly focused Gaussian beam scattering from narrow lymph micro vessels is presented.

5. CONCLUSIONS

The practice of teaching the described course shows that it is necessary to give the students a number of seminars aimed on the solution of some statistical tasks related to concrete examples of speckles applications in biomedicine. The set of common laboratory works has to be added by the following ones, utilizing the speckle technique:

- a. *Spermatozoa analysis*
- b. *Electrophoretic mobility of RBC*
- c. *Analysis of the process of bacteria colonies grow*

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Fig.1. Scheme of speckle-using vibrometer for biovibration monitoring

Fig.2. A sample of normal pulse wave signal registered on the wrist

Fig.3. Speckle microscope for lymph flow measurements in micro vessels

a

b

c

Fig.4. Intensity fluctuations spectra for diffraction of focused beam in different regions of the vessel:
a) beam diffraction at the left edge of the lymph flow;
b) beam diffraction at the central region of the lymph flow;
c) beam diffraction at the right edge of the lymph flow.