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A laser-spectroscopy system for fluorescent diagnostics and photodynamic therapy of diseases of eye retina and choroid

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***Abstract*. A laser-spectroscopy system for the fluorescent diagnostics and photodynamic therapy of pathologic eye-fundus changes combined with the use of the Photosens compound is developed. The system is tested on experimental animals (mice and rabbits).**

Keywords: *fluorescent diagnostics, photodynamic therapy, slit lamp, laser, subretinal neovascular membrane.*

It is known that the diseases of eye retina and choroid are the main reasons for poor eyesight and blindness. The subretinal neovascular membrane (SNM), appearing in the central zone of the eye fundus due to the invasion of new choriocapillary layer (eye-fundus choroid) vessels under the pigment epithelium or retina, complicates different inflammatory and dystrophic processes, thus resulting in a considerable and stable visual impairment. This pathology accounts for the diability in more than 80 % of cases [1].

Until recently, either the television optical angiography of the eye fundus with sodium fluorescein [2] or the fluorescent angiography with indocyanin green [3–7] was used to diagnose the SNM, while the laser coagulation [8] or the surgical intervention [9, 10] was applied as a treatment. However, these methods are not always efficient and may cause complications.

In this context, a new SNM therapy method using the photodynamic effect [11] is recently developed. This method is based on the photodynamic occlusion of newly grown vessels with the preservation of surrounding tissues (retina, pigment epithelium, choriocapillaries), and allows the selective action upon only pathologic areas, thus increasing the efficiency of treatment and, at the same time, improving the eyesight prognosis. To realise this method, the photosensitiser Visudin, representing the porphyrin derivative – verteporphyn (benzoporphyrin derivative monoacid), was developed in CIBA Vision (USA). Visudin does not fluoresce and, consequently, cannot be used for the SNM diagnostic. Therefore, the angiographic study of patients with the introduction of either sodium fluorescein or indocyanin green should be carried out to confirm the appearance of the SNM. Thus, the procedure of diagnostics and photodynamic therapy is divided into two separate-in-time stages. The realisation of the photodynamic therapy itself becomes also complicated due to the absence of fluorescence, and a doctor using a microscope and colour video camera has to proceed only from clinical signs, which substantially diminishes the efficiency of the therapy.

In this connection, we worked over the possibility to use the domestically produced photosensitiser Photosens [12] (FGUP State Research Centre 'NIOPIK', Moscow) remarkable for high efficiency of both the photodynamic effect and the fluorescence excited in the spectral range of 665–680 nm. This photosensitiser is allowed to be used in Russia for the fluorescent diagnostics and photodynamic therapy of neoplasms. At present, the possibility of its application in diagnostics and treatment of nononcological pathologies is studied.

The investigation of the Photosens pharmacokinetics in eye-fundus vessels of rabbits with the use of a LESA-01-BIOSPEK laser spectral-fluorescent analyser ('Biospek', Moscow) [13] showed (Fig. 1) that Photosens is almost completely removed from intact vessels of the eye in eight days. At the same time, owing to the penetration of Photosens through vessel walls, the Photosens depot is formed in the pathological locus of the SNM, and its concentration almost does not decrease in time because the photosensitiser comes out of the vascular system. These effects as well as the invasion of new vessels through the pigment epithelium, which strongly absorbs light in the 650–700-nm range, provide the high contrast between the pathological locus and intact areas of the eye fundus, and allow the sufficiently accurate determination of pathology bounds by means of the Photosens fluorescence pattern. Under the laser therapeutic exposure, the above-mentioned effects also facilitate the increase of the selectivity of photodynamic action and the preservation of surrounding tissues.

The aim of this work is to develop the laser-spectroscopy system for the fluorescent diagnostics and photodynamic therapy of pathalogic eye-fundus changes with the use of Photosens and to test these methods on experimental models of eye pathologies of animals (mice and rabbits).

The developed laser-spectroscopy system is based on the conventional ophthalmologic device – a ShchL-G3 slit lamp (joint-stock company 'ZOMZ'). In addition to the tradi-

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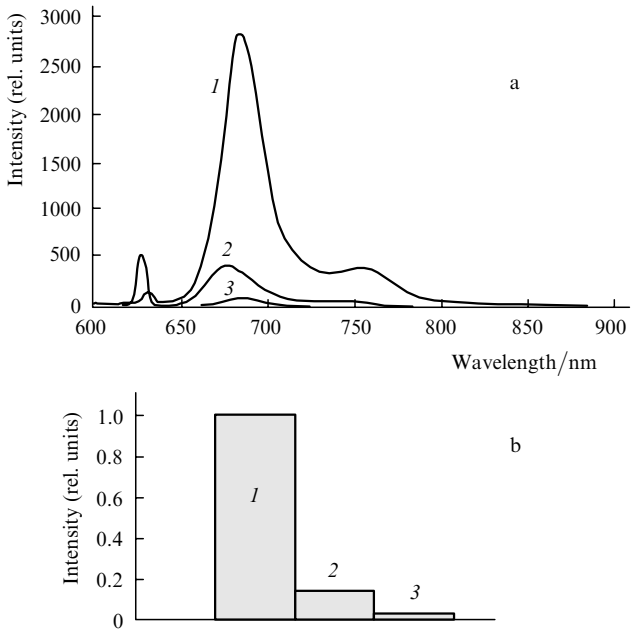

Figure 1. Spectra (a) and integrated intensity (b) of Photosens fluorescence in rabbit's eye-fundus vessels after an hour (*1*), three days (*2*), and eight days (*3*).

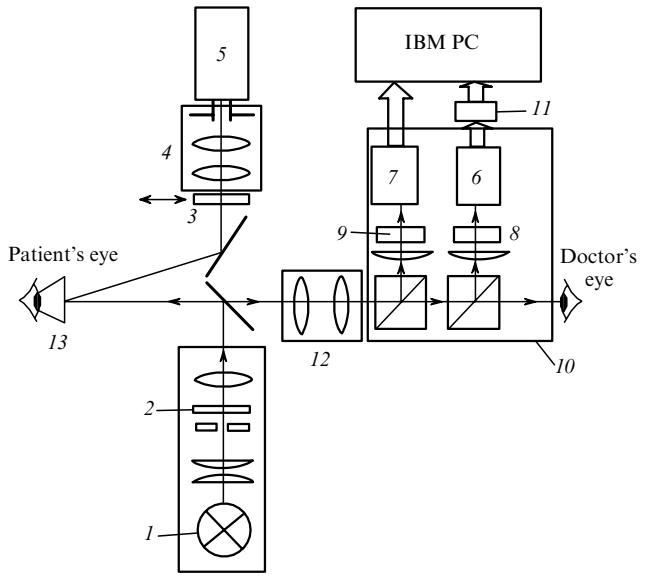

Figure 2. Scheme of the laser-spectroscopy complex for the fluorescent diagnostics and photodynamic therapy of eye-fundus pathologies: (*1*) slit-lamp illuminator; (*2*) filter of illuminator; (*3*) diverging lens; (*4*) optical adapter; (*5*) laser; (*6*) highly sensitive video camera; (*7*) colour video camera; (*8*) filter of highly sensitive video camera; (*9*) filter of colour video camera; (*10*) video channel; (*11*) noise reduction system of highly sensitive video camera; (*12*) microscope; (*13*) Goldman lens.

tional ophthalmologic methods, which were applied as a basis, it was necessary to realise a number of new functions allowing the study of the distribution of Photosens concentration in vessels of retina and surrounding tissues, and ensuring the local photodynamic effect on pathological locuses. This requirement needed a number of problems to be solved, including: (i) observing a weak fluorescent image in case of the limited exciting laser power density (in order not to initiate the photodynamic process during the diagnostics) under the intense white light exposure of the eye-fundus area being studied; and (ii) observing a colour image of the eye fundus (using slit illumination of the eye fundus by white light which is conventionally applied in such devices) with the eye-fundus area under the intense laser-radiation exposure.

When developing the system, the ShchL-G3 slit lamp was supplied with the video channel including the colour camera, high-sensitivity black-and-white camera, and personal computer for the processing and imaging of video information, as well as with the laser and optical adapter, focusing the laser radiation onto the eye fundus by means of a Goldman lens (Fig. 2). The diameter of the laser focal spot could be varied from 0.1 to 1 mm.

Since the ratio of the exciting radiation intensity to the fluorescence intensity is about $10^3 - 10^4$ (depending on the Photosens concentration), the optical system of the developed complex should provide the maximum transmission of the fluorescence to the highly sensitive video camera, almost excluding simultaneously the scattered laser radiation getting into the camera. Therefore, when choosing the components of the optical system we took into account the fact that the photodynamic effect and fluorescence of Photosens in the eye-fundus vascular system are most efficiently excited in a comparatively narrow (663–675 nm) band, while the fluorescence spectrum lies between 665 and 740 nm.

Based on these considerations, we used a special set of interference and absorption filters, which provided the transmission of only the fluorescent image in the 690–740-nm range to the video camera, whereas both the short-wavelength laser radiation scattered by eye tissues, and the long-wavelength radiation (above 740 nm) mainly determined by external illumination (Fig. 3) were totally suppressed. In addition, we used a filter, suppressing radiation with the wavelength above 640 nm (since it is not involved in forming the image by the colour camera) into the slit-lamp illuminator. Thus, we provided the protection of the video camera from the long-wavelength radiation of the illuminator. The analogous filter was also included in the optical system of the colour camera, thus

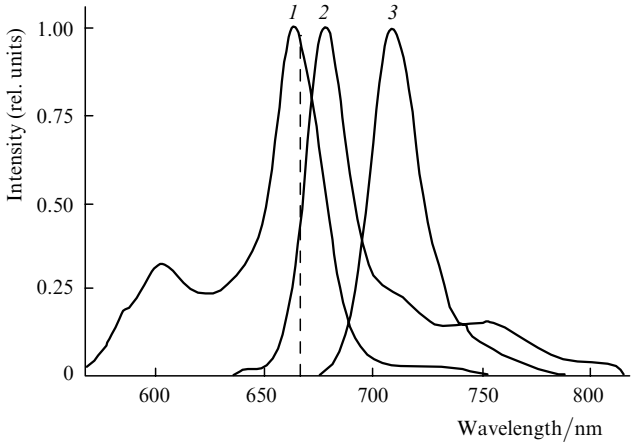

Figure 3. Fluorescence excitation (*1*) and fluorescence (*2*) spectra of Photosens, and transmission of the filter system of highly sensitive video camera (*3*); dashed line denotes the wavelength of the diode laser exciting Photosens.

allowing us to substantially lower the influence of the scattered laser radiation on the brightness and colour transfer of the colour image formed by this camera.

The fibre-output laser, developed for our system, provided the radiation power of up to 90 mW in the fibre of the 200-μm diameter and numerical aperture of 0.22. To achieve the maximum sensitivity of the system the laser wavelength was chosen within the range of 667–671 nm (the short-wavelength side of the absorption band). Taking into account the transmission of the optical adapter, focusing laser radiation onto the eye fundus (Fig. 4), and Goldman-lens losses, the power of radiation exposing the pathology could reach several milliwatts. This value of radiation power ensured the power density at least as high as 3 W cm^{-2} within the entire light-beam diameter adjustment range (100–1000 μm). In this case, the dose density of up to 300 J cm^{-2} was achieved within the exposure period not exceeding 90 s (the maximum time allowable for such ophthalmologic procedures). This dose density, in case of using Photosens, is quite sufficient for the photodynamic effect entailing the occlusion of vessels.

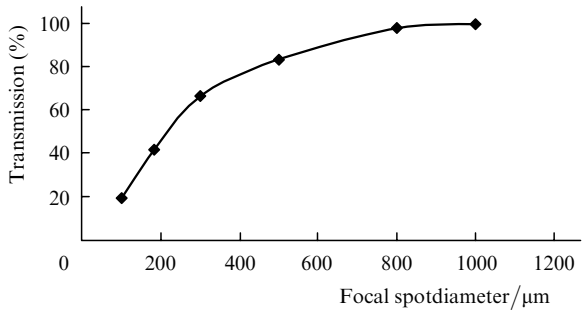

Figure 4. Light transmission of the optical adapter of the laser-spectroscopy system; radiation is coupled through the fibre of 200-μm diameter and numerical aperture of 0.22.

Since the power density of laser radiation used in the diagnostic regime is minimal (in order to avoid the photodynamic injury of intact vessels of the eye fundus), the fluorescence intensity is low. Therefore, in case of the camera operating with faint light fluxes, the white noise (the stochastic low-intensity high-frequency component of the camera video flux), observed as 'snow', should be suppressed to achieve a contrast observation of a fluorescent object, especially that of a small size. To realise the noise reduction, the frame-storage principle is usually employed, thus giving the possibility to improve the signal-to-noise ratio by $n^{1/2}$ in case of averaging the signal over n frames. Thus, for example, when averaging over 25 frames (the imaging lag in this case is 1 s) the signal-to-noise ratio can be improved by a factor of five. However, this method requires the excessive computer resources. In this connection, we proposed an optimal variant of a multiple-frame storage system allowing the minimisation of personal computer resources necessary for the noise elimination. In this system, each incoming frame (IF) of a video flux does not replace the previous frame, but modifies the current image (CI) by appending to it with a certain coefficient. The new image is formed by the pixel-by-pixel composition of the IF and CI with the coefficients of the IF and CI being chosen in such a way as to add up to unity. The resultant image is displayed on a monitor and serves as a CI for the next IF. Such a system allows one to substantially suppress the white noise of the video flux in the real-time operation mode.

The fluorescent test object with a periodic structure was required for the estimation of the sensitivity and spatial resolution of the system. Since Photosens does not fluoresce in a dry form, the necessary test object is made on the basis of the reticular capron fabric treated with the Photosens solution. Characteristic sizes of fabric elements approximately correspond to typical sizes of pathological focuses: the fabric cell size is 250–500 μm, the fibre bunch size is 0.1 mm, the size of a single fibre is about 20 μm. After processing and drying, the stable solid solution of aluminium sulfophthalocyanine in capron is formed in surface layers of capron fibres. This solid solution has the fluorescence characteristics close in intensity and spectrum to those of Photosens. The spectral characteristics of this solution are given in Fig. 5a. Fig. 5b shows the fluorescent image of the test object obtained by our system.

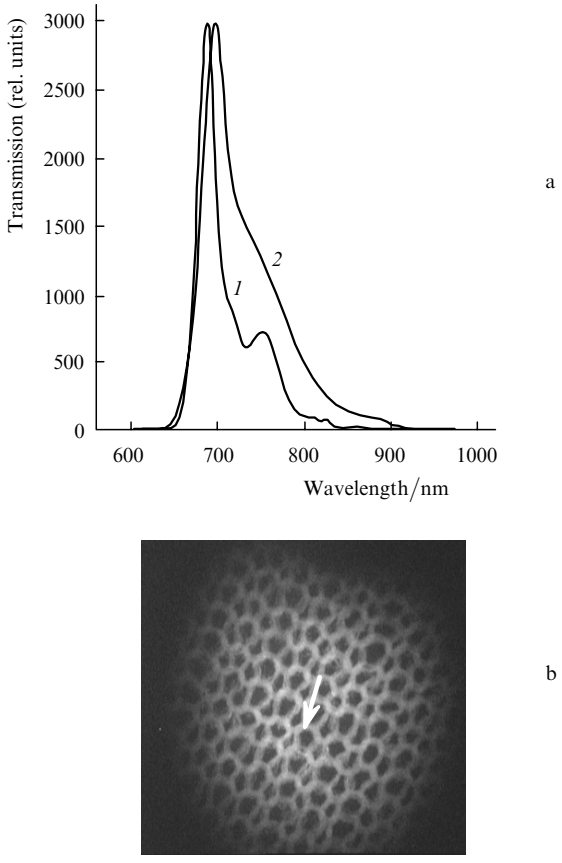

Figure 5. (a) Fluorescence spectrum of the Photosens solution with 10-mg L^{-1} concentration (*1*) and of the test object treated by this solution (*2*). (b) Fluorescent image of the test object; the arrow points at separate fibre of 20-μm diameter.

To estimate the safety of the use of Photosens and the developed system for the fluorescent diagnostics and photodynamical therapy, the tests on the experimental animals were carried out.

In the first group of animals, the influence of Photosens on eye tissues in case of exposure within the agent absorption band was studied. The compound was intro-

duced intravenously in doses of 0.01, 0.1, 0.3, and 0.5 mg kg^{-1}. Its concentration in eye-fundus vessels was controlled by means of the LESA-01-BIOSPEK spectral-fluorescent analyser during the following two weeks.

In the second group of animals, the laser exposure (the 500-mW cm^{-2} radiation power density for the 100-s exposure time) was applied without the preliminary introduction of Photosens.

In the third group of animals, the laser effect on eye vessels and tissues in the diagnostic regime (10 mW cm^{-2} during 100 s) for the same compound doses of 0.01, 0.1, 0.3, and 0.5 mg kg^{-1} was estimated. Fig. 6 shows the fluorescent image of rabbit's eye-fundus vessels obtained for the Photosens dose of less than 0.1 mg kg^{-1}. Such doses are too low and can be used only for diagnostic purposes.

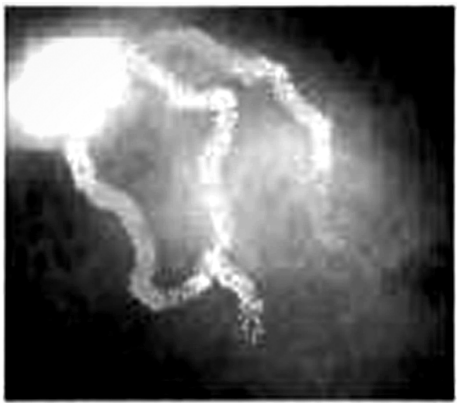

Figure 6. Fluorescent image of rabbit's eye-fundus vessels.

In the fourth group, the therapeutic laser exposure (the 500-mW cm^{-2} radiation power density for the 90-s exposure time) was applied after the intravenous Photosens introduction in doses of 0.01, 0.1, 0.3, and 0.5 mg kg^{-1}.

The animals of all groups were euthanatised right after the experiment, in a week, and in two weeks; eyes were extracted for the morphological study. Histological studies, realised through methods of light and electron microscopy, did not reveal any changes in eyes of the first three groups of animals. In the fourth group, the lesion of the rear eye segment arouse only in the laser focal spot, while the extent of the laser effect depended on the dose of the introduced compound and the time of exposure.

The conducted experiment allowed us to make a conclusion that both Photosens as itself (without the laser exposure) and the isolated laser action without sensitisation have no harmful effect on eye tissues. The exposure of the eye in the diagnostic regime after the introduction of Photosens in 0.01, 0.1, 0.3, and 0.5-mg kg^{-1} doses is also innocuous. The lesion of the rear eye segment in the therapeutic regime occurs only within the laser focal spot after the preliminary introduction of photosensitiser, while the extent of the lesion depends on the agent dose and the time of exposure.

Thus, the precision and sensitivity of the developed system make it possible to observe the fluorescence of studied eye pathologies in case of compound concentrations by order of magnitude lower than those used for oncological diseases. Dimensions of pathologies reach 100–200 μm, which is sufficient to be resolved by the visualisation system and the system of the laser radiation delivery. In this context, we expect a successful combined application of the developed system and the Photosens agent for the treatment of such complicated diseases as the SNM, glaucoma and others, as well as a quick and wide spread of this equipment and method in clinical practice. The more so as the price of the equipment and the agent is ten times lower than that of their western analogues. The start of clinical trials of the technique is scheduled for the end of this year.

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