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MODELING OF THE AUTOFLUORESCENCE SPECTRA OF THE CRYSTALLINE LENS WITH CATARACT TAKING INTO ACCOUNT LIGHT SCATTERING

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The model of the autofluorescence spectrum formation of a crystalline lens taking into account light scattering was presented. Cross sections of extinction, scattering and absorption were obtained numerically for models of normal crystalline lens and cataract according to the Mie theory for polydisperse systems. To validate the model, data on the autofluorescence spectra of the normal lens and cataracts were obtained using an experimental ophthalmologic spectrofluorometer with excitation by UV light emitting diodes. In the framework of the model, the influence of the lens light scattering on the shape of the luminescence spectrum was estimated. It was found that the changes in the fluorescence spectrum of lenses with cataracts can be completely interpreted by the light scattering.

Keywords: *fluorescence, light scattering, cataract maturity.*

Introduction. Fluorimetry of the eye is a promising technique for research and diagnosis in ophthalmology [1–3]. A number of ophthalmic fluorophotometers have been proposed to assess the fluorescence of the eye tissues. One of the applications of fluorescent methods in ophthalmology is the diagnosis of cataracts [4–6]. A cataract is a chronic degenerative disease manifested by partial or complete clouding of the lens of the eye, which affects up to 50% of people over the age of 70 [7–9]. In clinical practice in Russia, most commonly classification is based on the main localization of opacities: cortical and nuclear cataracts. Classification according to the degree of cataract maturity is also common [8]: initial, immature, mature and hypermature. The international classification of cataracts LOCS takes into account the delicate color gradations of not only the nucleus of the lens, but also its cortical structure [4]. The lens is ~40–70% protein in composition. The clarity of the lens is normally determined by the location of spherical proteins (~17 nm in diameter) on the membranes of fibers [7]. An increase in the proportion of water-insoluble proteins accompanies cataractogenesis in nuclear cataracts [7–9]. Insoluble proteins have the ability to sorb on membranes. The development of cataracts is associated with genetic causes (most often, the mutation of 10 genes encoding crystallin proteins, a mutation of genes encoding connexins, cytoskeleton proteins, or other membrane-associated proteins or regulators of protein expression with chaperone activity) and factors provoking oxidative stress, accumulation of protein glycation end-products, and often accompanies the natural aging process, the progression of diabetes, neurodegenerative diseases, and chronic toxication, immunodeficiencies, etc. [7, 9]. In nuclear cataract lenses more than 90% of insoluble protein sulfhydryl groups are in the oxidized state [9], which also enhances the fluorescence yield. Lens proteins (crystallins) are synthesized by epithelial cells during the growth of the organism, but the descendants of epithelial cells lose the ability to synthesize proteins, therefore the structure of synthesized crystallins is to be maintained throughout the entire subsequent life of the organism [7–9]. That is why the lens transparency is determined by the protein conformation stability, especially of the β- and γ-crystallins, which can be disturbed by their oxidation (of tryptophan, cysteine, methionine residues), deamidation (of glutamine and asparagine residues), glycation (including non-enzymatic in case of chronic hyperglycemia), fragmentation, accumulation of homocysteine in the tissue. These changes are normally recognized by the α-crystallins of the lens, which constitute ~50% of the total mass of the lens proteins, are small heat shock proteins and exhibit chaperone activity (contribute to the restoration of the initial conformation of damaged structural proteins). Also, in the course of aging there is an increase in brown coloration of the lens nucleus, which leads to

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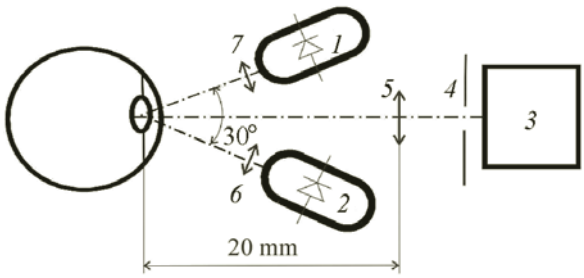

Fig. 1. Optical diagram of the spectrofluorometer with excitation by UV LED: *1*, *2* — UV LEDs (λ_{max} = 345 ± 5 nm); *3* — spectrometer; *4* — spectrometer aperture; *5* – condenser lens; *6*, *7* — focusing lenses of excitation LEDs.

an increase in the absorption of UV light. UV radiation in turn stimulates the accumulation of active forms of oxygen and the formation of covalently bound high-molecular aggregates: the so-called "yellow protein" containing a yellow pigment covalently bound to the age-related protein (AP) begins to accumulate in the nucleus of the lens [7–9]. The appearance of such large (>500 nm) structures and aggregates leads to increased light scattering, deterioration of the optical properties of the lens, and cataract development [7, 9].

Thus, clouding of the lens in case of cataracts is promoted by denaturing and aggregation of structural proteins, as well as by inadequate functioning of lens chaperones or components of the antioxidant system. Taking into account that the work of the latter is largely determined by the bioavailability of pyridine and flavin nucleotides (NAD(P)H, FAD), the evaluation of the lens autofluorescence spectrum in the range corresponding to the optical characteristics of these endogenous fluorophores is a convenient method for screening and assessment of the state of the lens.

The main fluorophores of the lens are tryptophan, 3-hydroxy-L-kynurenine-O-β-glucoside (3-HKG), flavins, ascorbic acid, NADH, NADPH, β-carboline, anthranilic acid, etc. [4, 5, 7–9]. The ratio of these fluorophores changes with the development of cataracts. Light scattering [1, 4] has a significant effect on the formation of the fluorescence spectrum of the lens. Various theoretical methods have been used to describe light scattering by biological tissues [10–14]. In our opinion, the most promising method for such model biomedical light scattering calculations is the rapidly developing numerical finite-difference time-domain method (FDTD) [10, 11], which can be used for arbitrary nonspherical, multilayer and other biological cells and tissues. FDTD is superior in accuracy to methods based on the solution of the equation of radiative transfer (ERT) [11, 12]. However, high computer resource allocation is required for high accuracy of FDTD.

In this paper, an alternative method for modeling the lens (and its constituent proteins) is employed using an ensemble or a system of polydisperse spheres (SPS) [13]. This method is less costly, and optical equivalence of the SPS and an ensemble of chaotically oriented ellipsoids has been proven [14]. In addition, analytical formulas [14–16] in the Rayleigh–Hans–Debye and anomalous diffraction approximations have been obtained for SPS, which gives an additional opportunity to control conclusions and calculations at any stage. Of course, the adequacy of the SPS lens model is somewhat inferior to the FDTD method, but it is not inferior to models based on the ERT.

The purpose of this work is to estimate the contribution of light scattering to the formation of the autofluorescence spectrum in the development of cataracts.

Experiment and Calculations. Experimental fluorescence spectra of the lens were obtained with the help of a small-size ophthalmic spectrofluorometer with excitation by UV light emitting diodes (LED) [3, 6]. The optical diagram of the spectrofluorometer is shown in Fig. 1. Spectra of the lens of patients (15 spectra — control, age 20–25 years, without pathologies of vision; 30 spectra — mature cataract) were obtained *in vivo*, corrected for Rayleigh scattering of the exciting radiation, normalized to the maximum intensity and averaged over the samples. After treatment, the relative spread of fluorescence intensity in the control group was 2%, and in the group with mature cataracts — 5%.

For model calculations of light scattering, a model of a healthy and “transparent” lens and a model of a turbid lens with a cataract in the form of a SPS proposed earlier [13, 17, 18] were used. Calculations were made using the Mie theory, the algorithm described in [19] and its modifications for the efficiency factors of the attenuation, absorption, and light scattering of a spherical particle in an absorbing medium [20] and generalizations for a SPS [21]. In contrast to [17, 18], integration was carried out using Gauss–Legendre quadratures with an accuracy of ≤1%.

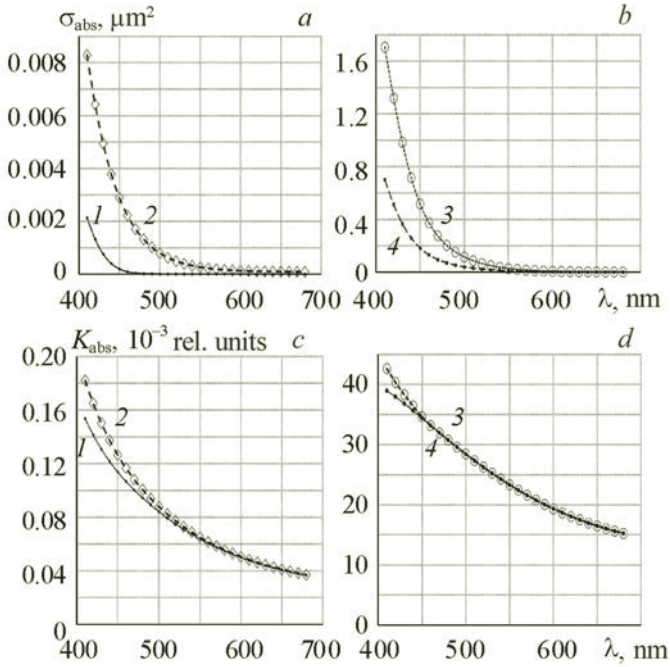

Fig. 2. Dependence of the cross section (a, b) and the absorption coefficient (c, d) on the wavelength for models of a normal lens in a young (1), elderly (2) person, in a mature (3) and initial (4) cataract.

A model of the normal law of polydisperse spheres with $r_0 = 0.25$ μm and $\sigma = 0.02$ μm with a relative refractive index $m = 1.03$ immersed in an aqueous medium was used for a healthy lens [13]:

$$f(r) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left[-\frac{(r - r_0)^2}{2\sigma^2}\right], \quad (1)$$

and in the case of a cataract, for a cloudy lens the gamma-distribution of polydisperse spheres with $r_0 = 1.0$ μm, $\mu = 20$ [13] was:

$$f(r) = r^{\mu} \exp(-\mu r/r_0) . \quad (2)$$

The data on the dependence of the refractive index of the lens on the wavelength were taken from [22]. The imaginary part of the refractive index of the lens $\chi(\lambda)$ consists of three components, as in [17, 18]:

$$m = n(\lambda) + i\chi(\lambda) = n(\lambda) + i[t\chi_t(\lambda) + k\chi_k(\lambda) + p\chi_p(\lambda)] , \quad (3)$$

where $0 < t + k + p < 1$, the relative proportions of chromophores of the lens in absorption: t — tryptophan, k — 3-HKG, p — age-related protein (AP).

In the calculations, four models were used for the refractive index of the lens tissue: the norm for the "young" — $t = 0.003$, $k = 0.005$, $p = 0$; the norm for the "elderly" — $t = 0.003$, $k = 0.002$, $p = 0.015$; mature cataract — $t = 0.003$, $k = 0.03$, $p = 0.03$; initial cataract — $t = 0.003$, $k = 0.01$, $p = 0.01$.

Results and Discussion. Wavelength dependences were calculated for the cross sections of scattering $\sigma_{sc} = \sigma_{sc}(\lambda)$, absorption $\sigma_{abs} = \sigma_{abs}(\lambda)$, extinction $\sigma_{ext} = \sigma_{ext}(\lambda)$ and backscattering $\sigma_b = \sigma_b(\lambda)$ by polydisperse spherical particles for models of the normal lens of a young and elderly person and for models of a lens with mature and initial cataracts. Figure 2a and b shows the wavelength dependence of the absorption cross-sections $\sigma_{abs} = \sigma_{abs}(\lambda)$. For the case of coherent extinction, the absorbance spectrum of the lens K_{abs} was calculated [17] at an average bulk density of the scattering lens particles $\rho = 10^{12}$ m^{-3} (Fig. 2c and d):

$$K_{abs} = 1 - T = 1 - \exp(-\rho\sigma_{ext} d) , \quad (4)$$

where σ_{ext} is the cross section for extinction; d is the characteristic thickness of the lens (5 mm), and T is transmittance.

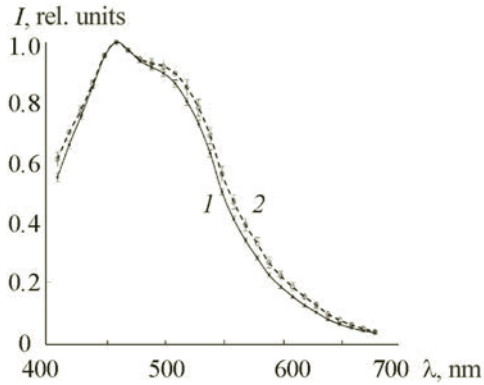

Fig. 3. Experimental luminescence spectrum with UV LED excitation in a normal lens (1) and in a lens with a mature cataract (2).

The average normalized fluorescence spectra of the lens obtained in an experiment with excitation by UV LED are shown in Fig. 3. There is some increase in the width of the fluorescence contour in mature cataracts. Among the main fluorophores of the lens [18], which have a large quantum yield, 3-HKG contributes the most to the fluorescence spectrum upon excitation at λ_{max} = 345 nm. Since the sources of fluorescence are inside the lens, in a mature cataract less extinction is observed in the long-wavelength fluorescence region.

An important methodological issue of optical fluorescence spectroscopy of turbid media is the evaluation of light scattering effects on the shape of the fluorescence spectrum. Taking into account the calculated transmittance T (see (4)) and the reflectance R (normalized cross section of backscattering σ_b) of the lens in a normal lens and in case of a cataract, a true fluorescence spectrum can be reconstructed.

As the simplest model of the lens, we take a plane layer, where luminescence arises, which, upon exiting the layer, is attenuated by the scattering of light forward and amplified by backscattering:

$$I_1 = I_0/T(1 + qR)\,, \tag{5}$$

where I_0 and I_1 are the luminescence intensities at the entrance and exit of the lens material layer; R is the reflection or backscattering coefficient, and q is the relative efficiency factor for the collection of backscattering radiation ($0 \le q \le 1$).

Suppose that changes in the fluorescence spectrum in the development of cataracts are associated with a change not so much in the ratio of fluorophores as in the scattering of light in the lens. Then the function $F = F(\lambda)$ depends only on the light scattering characteristics of the lens:

$$I_C = FI_N\,, \tag{6}$$

where I_C and I_N represent the luminescence intensity in case of a cataract and in a normal lens. Using (5), we can calculate the theoretical function F (the ratio of fluorescence intensities of the lens in in case of a cataract and in a normal lens):

$$F = \frac{I_C}{I_N} = \frac{T_C\left(1+qR_C\right)}{T_N\left(1+qR_N\right)}, \tag{7}$$

where T_C and T_N and R_C, R_N are the transmittance and reflectance of the lens in case of a cataract and in a normal lens.

The experimental functions F (ratios normalized to λ = 540 nm, fluorescence intensities of the lens in case of a cataract and in a normal lens) were compared with UV LED and laser excitation using experimental data [5] and model spectra F calculated from Eq. (7). Figure 4 shows a close coincidence of the model and experimental spectra at an average bulk density of the scattering centers attributed to the total thickness of the lens, $\rho = 8\cdot10^{12}$ m^{-3} and q = 0.5 for fluorescence excitation by LED and $\rho = 36\cdot10^{12}$ m^{-3} and q = 1 for excitation by laser. The difference in the mean bulk densities of the scattering centers ρ for laser and LED excitation is associated with the optical excitation scheme (frontal for a laser spectrofluorometer and, for a spectrofluorometer with UV LEDs, intersecting at a 30^o angle), coherence of radiation in a laser setup forming a speckle field of fluorescence excitation and, respectively, a difference in the effective depth of penetration of the exciting radiation into the lens. The difference in q can be explained by different numerical apertures collecting fluorescence in optical systems of the two spectrofluorometers. So, for a laser system with a light-guide delivery NA = 0.2, and for an LED system NA = 0.1.

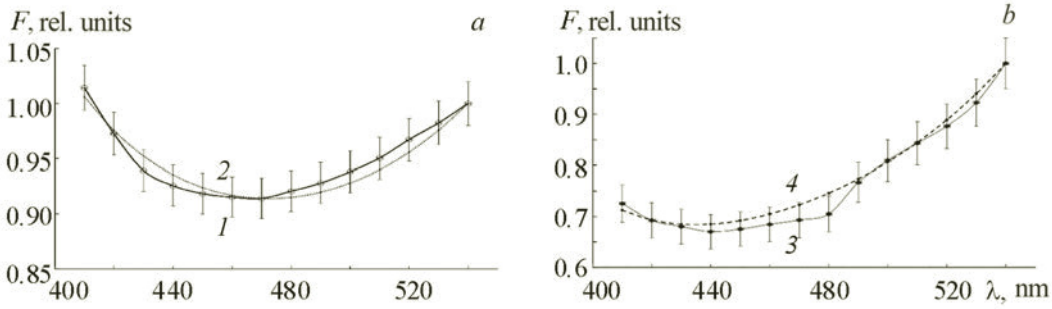

Fig. 4. The ratio of fluorescence intensities of a lens with mature cataracts and in a normal lens F as a function of wavelength for spectrofluorometers with UV LED (a) and laser excitation (b) in an experiment (1, 3) and in a model (2, 4) at $\rho = 8 \cdot 10^{12}$ m^3 and $q = 0.5$ (a), $\rho = 36 \cdot 10^{12}$ m^3 and $q = 1$ (b).

Thus, changes in the shape of a fluorescence spectrum in lenses with cataracts within the experimental error (regardless of the fluorescence excitation method – by UV LED or laser) can be obtained only by introducing other light scattering parameters in cataracts. However, the problem of the ratio of fluorophores in a mature cataract remains, since, for example, there are non-tryptophan fluorophores not associated with light-scattering proteins [23].

Conclusions. Attenuation, scattering and absorption cross section spectra for lens models in the form of a system of polydisperse spheres in a normal lens and in one with a cataract were calculated. Using the lens model in the form of a flat layer, the degree of influence of light scattering of the lens on the shape of the luminescence spectrum was evaluated. Major changes in the shape of the fluorescence spectrum of crystalline lenses with cataracts can be fully interpreted by light scattering.

REFERENCES

1. J. Burd, S. Lum, F. Cahn, and K. Ignotz, *J. Diabetes Sci. Technol.*, **6**, No. 6, 1251–1259 (2012).
2. M. Klemm, D. Schweitzer, S. Peters, L. Sauer, M. Hammer, and J. Haueisen, *PLoS One*, **10**, No. 7 (2015) e0131640; doi:10.1371/journal.pone.0131640.
3. A. A. Topakova, V. V. Salmin, V. V. Gar′kavenko, J. S. Levchenko, and V. I. Lazarenko, *Proc. SPIE*, **9917** (2016) 991715(1–4); doi: 10.1117/12.2229816.
4. S. Siik, *Acta Ophthalmol. Scand.*, **77**, No. 5, 509–514 (1999).
5. E. S. Vladimirova, V. V. Salmin, A. B. Salmina, S. A. Oskirko, V. I. Lazarenko, and A. S. Provorov, *Zh. Prikl. Spektrosk.*, **79**, No. 1, 136–140 (2012) [E. S. Vladimirova, V. V. Salmin, A. B. Salmina, S. A. Oskirko, V. I. Lazarenko, and A. S. Provorov, *J. Appl. Spectrosc.*, **79**, 126–130 (2012)].
6. V. Salmin, V. Gar′kavenko, J. Levchenko, D. Skomorokha, E. Vladimirova, A. Solovieva, A. Topakova, and V. Lazarenko, *Asia Communications and Photonics Conference 2014*, OSA Technical Digest (online), Optical Society of America (2014), ATh3A.203; doi:10.1364/ACPC.2014.ATh3A.203.
7. H. Davson, *Physiology of the Eye*, Academic Press, New York & San Francisco (1980), pp. 116–164.
8. V. G. Kopaeva, *Eye Diseases* [in Russian], Meditsina, Moscow (2002), pp. 245–268.
9. L. N. Makley, K. A. McMenimen, B. T. DeVree, J. W. Goldman, B. N. McGlasson, P. Rajagopal, B. M. Dunyak, T. J. McQuade, A. D. Thompson, R. Sunahara, R. E. Klevit, U. P. Andley, and J. E. Gestwicki, *Science*, **350**, No. 6261, 674–677 (2015); doi:10.1126/science.aac9145.
10. R. Drezek, A. Dunn, and R. Richards-Kortum, *Opt. Express*, **6**, 147–157 (2000).
11. L. Marti-Lopez, J. Bouza-Dominguez, J. C. Hebden, S. R. Arridge, and R. A. Martinez-Celorio, *J. Opt. Soc. Am. A*, **20**, No. 11, 2046–2056 (2003).
12. D. A. Rogatkin, *Med. Tekh.*, No. 2, 10–16 (2007).
13. A. V. Priezzhev, V. V. Tuchin, and L. P. Shubochkin, *Laser Diagnostics in Biology and Medicine* [in Russian], Nauka, Moscow (1989), pp. 68–74.
14. L. E. Paramonov, *Opt. Spectrosc.*, **112**, No. 5, 787–795 (2012); doi: 10.1134/S0030400X1205013X.

15. M. A. Box, S. Y. Lo, B. H. J. McKellar, and M. Reich, *Quart. J. R. Met. Soc.*, **104**, 959–969 (1978).
16. K. A. Shapovalov, *Opt. Atmos. Okeana*, **6**, No. 11, 1411–1415 (1993).
17. V. V. Tuchin and D. M. Zhestkov, *Proc. SPIE*, **3053**, 123–128 (1997); doi: 10.1117/12.266240.
18. D. M. Zhestkov, I. L. Maksimova, and V. V. Tuchin, *Proc. SPIE Ophthalm. Technol. VIII*, **3246**, 299–306 (1998); doi: 10.1117/12.309445.
19. K. Boren and D. Khafmen, *Absorption and Scattering of Light by Small Particles* [Translated from English], Mir, Moscow (1986), pp. 107–164, 602–608.
20. Q. Fu and W. Sun, *Appl. Opt.*, **40**, No. 9, 1354–1361 (2001).
21. M. I. Mishchenko, L. D. Travis, and A. A. Lacis, *Scattering, Absorption, and Emission of Light by Small Particles*, Cambridge Univ. Press, Cambridge (2002), pp. 115–190.
22. J. G. Sivak and T. Mandelman, *Vision Res.*, **22**, 997–1003 (1982).
23. Y. Shirao, E. Shirao, T. Iwase, A. Inoue, and S. Matsukawa, *Jpn. J. Ophthalmol.*, **44**, 198–204 (2000).