

BIOPHOTONICS

https://doi.org/10.1070/QEL16141

Ecophotonics: assessment of temperature gradient in aquatic organisms using up-conversion luminescent particles

E.K.Volkova, I.Yu. Yanina, A.P. Popov, A.V. Bykov, A.N. Gurkov, E.V. Borvinskaya, M.A. Timofeyev, I.V. Meglinski

Abstract. **In the frameworks of developing ecophotonics, we consider the possibility of applying luminescence spectroscopy for monitoring conditions of aquatic organisms, aimed at the study and prognosis of the effect of human activity and climate changes on the environment. The method of luminescence spectroscopy in combination with anti-Stokes luminophores (up-conversion particles) used as optical sensors is used for the noninvasive assessment of the temperature gradient in the internal tissues of aquatic organisms. It is shown that the temperature dependence of the intensity ratio observed in the maxima of the luminescence spectrum bands of the particles Y_2O_3:Yb, Er, administered in a biological object, is linear. This fact offers a possibility of using the up-conversion particles for assessing the metabolic activity of different tissues, including those in the framework of ecological monitoring.**

Keywords: *ecophotonics, up-conversion luminescent particles, luminescence spectroscopy, luminophores, environment monitoring, stress effect, aquatic organisms.*

1. Introduction

To date more and more attention is paid to the effect of global climatic changes on the environment. This effect is expected to increase essentially in the nearest future [1, 2]. The results of computer simulations predict an increase in the Earth temperature by 1.8–5 °C to 2100, which in turn will be accompanied by an essential increase in local temperature variations [2].

E.K.Volkova, I.Yu. Yanina N.G. Chernyshevsky Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia; University of Oulu, Optoelectronics and Measurement Techniques Laboratory, P.O. Box 4500, 90014 University of Oulu, Oulu, Finland;
A.P. Popov, A.V. Bykov ITMO University, Kronverkskii prosp. 49, 197101 Saint-Petersburg, Russia; University of Oulu, Optoelectronics and Measurement Techniques Laboratory, P.O. Box 4500, 90014 University of Oulu, Oulu, Finland;
A.N. Gurkov, M.A. Timofeyev Irkutsk State University, ul. Karla Marksa 1, 664003 Irkutsk, Russia;
E.V. Borvinskaya Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, ul. Pushkinskaya 11, 185910 Petrozavodsk, Russia; Irkutsk State University, ul. Karla Marksa 1, 664003 Irkutsk, Russia;
I.V. Meglinski ITMO University, Kronverkskii prosp. 49, 197101 Saint-Petersburg, Russia; Irkutsk State University, ul. Karla Marksa 1, 664003 Irkutsk, Russia; University of Oulu, Optoelectronic and Measurement Techniques Laboratory, P.O. Box 4500, 90014 University of Oulu, Oulu, Finland; e-mail: igor.meglinski@oulu.fi

Received 22 May 2016; revision received 22 December 2016
Kvantovaya Elektronika **47** (2) 153–157 (2017)
Translated by V.L. Derbov

The enhancement of the negative effect on the environment facilitates the reduction of stress resistance in aquatic organisms due to the activation of physiological and biochemical protection mechanisms in them [3]. In this case, there is a high risk that the species with a broad tolerance range, which are more stress-resistant, will force the less stress-resistant species out from their usual habitat. The change of species composition of aquatic ecosystems disturbs the ecological stability, which can have unpredictable consequences for the hydrologic system [4, 5].

In the course of adaptation to the habitat changes, specific protection mechanisms are developed in most freshwater organisms [6–8]. In the ecosystems of ancient freshwater lakes, the species dominate that have narrow adaptation to the specific conditions of the habitat and are particularly sensitive to the changes associated with the global warming and the rise of the environment temperature. These species are particularly subject to the risk of local elimination or essential reduction of the population number. In this connection, new methods of diagnostics are required [9] for prognosis of climatic change consequences, as well as for understanding the principles of protection mechanisms, elaborated by aquatic organisms.

In recent years, the methods of photonics, which is one of the most interesting and rapidly growing fields of high technology [10], find more and more applications in different spheres. This branch of science offers novel methods of noninvasive diagnostics at the expense of acquiring earlier unknown information about the structure of the studied medium at a micro- and macrolevel. The biophotonics offers unique possibilities for creating principally new noninvasive methods for the diagnostics of living systems with application to a wide scope of medical studies. The advantages of biophotonics and numerous examples of using the optical/laser methods for the diagnostics of living systems are summarised and generalised, e.g., in Refs [11, 12].

In the present paper, by the example of luminescence spectroscopy using anti-Stokes luminophores as optical sensors, we demonstrate that the application of photonics to ecological monitoring is possible in principle.

2. Materials and methods

The principles and fundamentals of luminescence spectroscopy are well known and thoroughly described by many authors [13]. To observe the photoluminescence signal from the anti-Stokes luminophores embedded in a biological tissue, we constructed an experimental setup, schematically presented in Fig. 1.

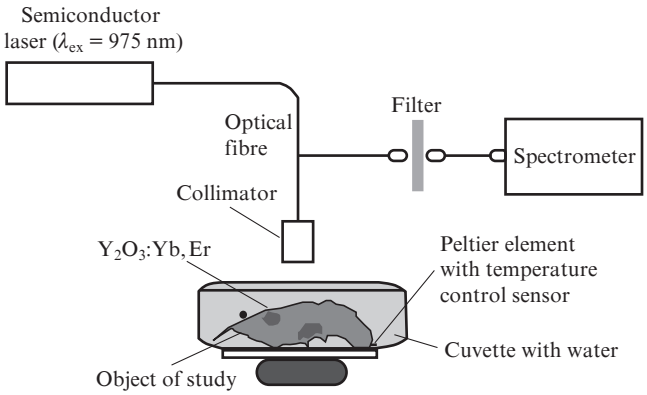

Figure 1. Schematic of the experimental setup.

The luminescence of particles was excited by a semiconductor ITC 4005 laser (Thorlabs, USA) at the wavelength λ_{ex} = 975 nm with a maximal pump power of 200 mW. The spectra of luminescence were measured at the distance 25 mm from the surface of the studied biological sample by means of a fibre optical sensor equipped with a collimator and an optical FB600-10–∅1" bandpass filter (Thorlabs, USA) blocking the radiation at the wavelength λ_{ex}. The photoluminescence signals were recorded by a CCS200 spectrometer (Thorlabs, USA). The acquisition time of the detected signal amounted to 200 ms.

The studies were carried out in four adult specimens of decapods Caridina multidentata (Amano shrimp) *in vivo*. The aqueous solution of up-conversion particles (5 μL with the concentration 0.7 μg μL^{-1}) was administered immediately before the experiment into the front part of the abdominal cavity filled with blood (haemolymph), the circulation of which all over the organisms is provided by the heart. For performing the experiment, the decapod was placed in a cuvette with water. The temperature t of water in the cuvette varied from 21 °C to 30 °C with a step 1 °C by means of the Peltier element equipped with a temperature control sensor. In all experiments we used the anti-Stokes luminophores, the up-conversion particles Y_2O_3:Yb, Er PT660-UF (Phosphor Technology, Great Britain) having a mean size of 1.6 μm (Fig. 2). To prevent the aggregation of the particles, the aqueous solution of up-conversion particles was treated with ultrasound immediately before the injection.

The photoluminescence excitation is schematically illustrated in Fig. 3a. The Yb^{3+} (donor) ions possess high effective absorption cross section in the IR range. The Er^{3+} (acceptor) ion poorly absorbs the exciting radiation but has a large number of electronic states, in which the ions can stay during a rather long time. Since the energy states of the acceptor ion are characterised by a long lifetime of metastable states, the donor ion can at once non-radiatively transfer the energy of several absorbed photons to the acceptor. As a result, the donor energy increases that causes the reduction of the photoluminescence wavelength [14, 15]. Under the light exposure, the donor ion absorbs a photon and undergoes transition to the excited state $^2F_{5/2}$ (Fig. 3a). Then the electron relaxes to the ground state $^2F_{7/2}$ and non-radiatively transfers the energy to the nearest acceptor ion, which undergoes transition to the state $^4I_{11/2}$.

Then in the course of the energy transfer from Yb^{3+} to Er^{3+} the transition of the acceptor to the level $^4F_{7/2}$ occurs, followed by the non-radiative relaxation to the levels $^4S_{3/2}$ and

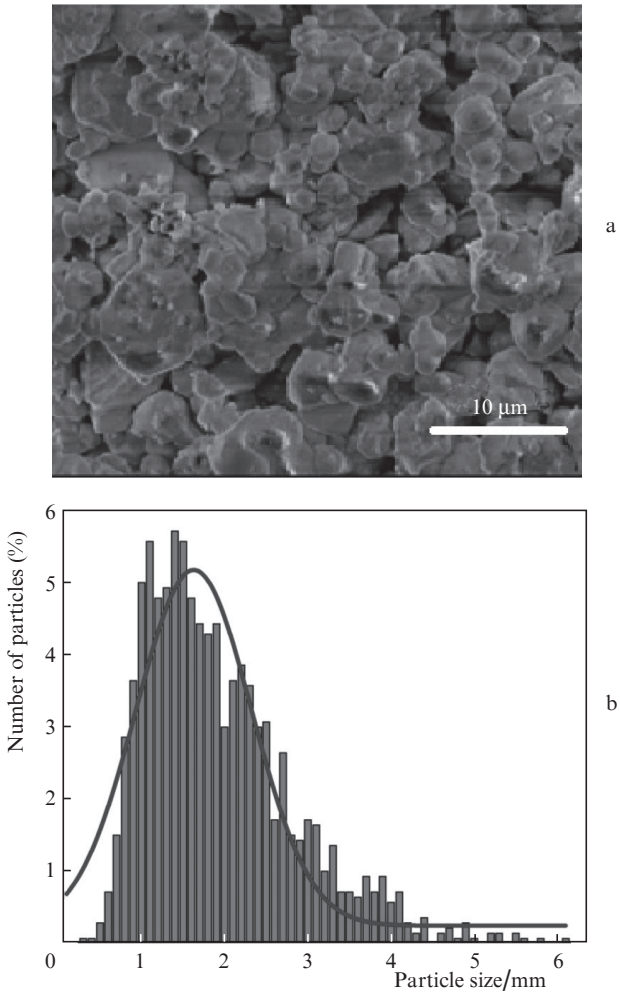

Figure 2. (a) Microphotograph of anti-Stokes luminophores Y_2O_3:Yb,Er, obtained by means of scanning electron microscopy and (b) the histogram of particles size distribution, obtained by processing the images of 1500 particles.

$^2H_{11/2}$. Finally, due to the transition to the ground state, the luminescence in the green part of the spectrum arises (Fig. 3a). In the case of the transition of Er^{3+} from the state $^4F_{9/2}$ to the ground state the luminescence in the red spectral region arises [14, 15]. In the red region, they usually select the transition with a maximal intensity, rather than all luminescence bands. The fine structure of the green region is also presented illustratively.

If the transfer of energy or the absorption by the acceptor ion from the excited state occurs during the time that is smaller than the lifetime of the state $^4I_{11/2}$ of the Er^{3+} ion, then the acceptor undergoes transition to the state $^4F_{7/2}$, from which it non-radiatively relaxes to the state $^4S_{3/2}$ and then to the ground state, emitting a photon in the green part of the spectrum. If, on the contrary, the lifetime of the metastable state $^4I_{11/2}$ is smaller than the time required for the secondary energy transfer, then the Er^{3+} ion undergoes transition to the more long-lived state $^4I_{13/2}$. In this case, the subsequent energy transfer from the donor will transmit the acceptor to the state $^2F_{9/2}$, from which it relaxes to the ground state with radiation in the red spectral region.

Thus, the radiation of up-conversion particles (Fig. 3b) in the green spectral region consists of five luminescence bands: 520 – 540 nm for the level $^2H_{11/2}$ (with the maxima at the wave-

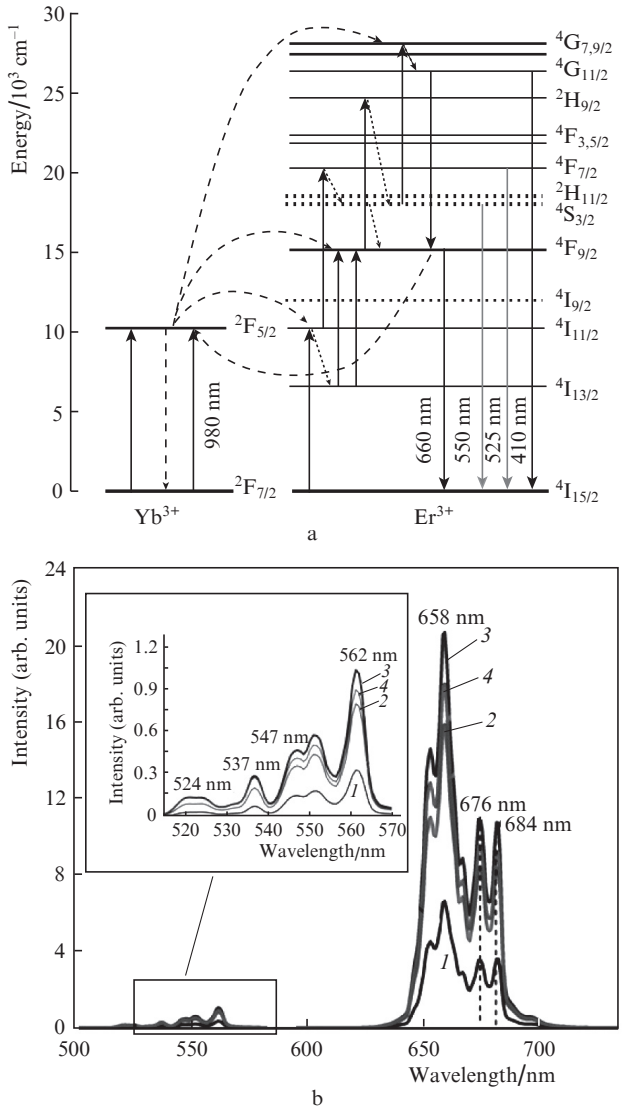

Figure 3. (a) Energy level diagram in the system of Yb^{3+} and Er^{3+} ions and (b) luminescence spectra of up-conversion particles (λ_{ex} = 975 nm) for the temperatures t = (*1*) 25 °C, (*2*) 35 °C, (*3*) 45 °C, and (*4*) 55 °C.

lengths 523 and 537 nm) and 540–570 nm for the level $^4S_{3/2}$ (with the maxima at the wavelengths 547, 551, and 562 nm). The radiation of up-conversion particles in the red spectral region consists of five luminescence bands with the maxima at 653, 658, 661, 676 and 684 nm.

For quantitative analysis of temperature variations in the present work, we use the luminescence intensity values at the wavelengths λ_1 = 676 nm and λ_2 = 684 nm. The choice of the intensity ratio at these wavelengths is caused by the maximal sensitivity to the variation of the environment temperature, since in this case the thermal equilibrium of populations of closely spaced excited levels, described by the Boltzmann distribution, is conserved [16]:

$$I_1(\lambda_1)/I_2(\lambda_2) = C\exp(-\Delta E/kT), \quad (1)$$

where $I_1(\lambda_1)/I_2(\lambda_2)$ is the ratio of intensity peaks in the luminescence spectrum at the wavelengths λ_1 and λ_2; C = const is the normalisation factor determined by the multiplicity of degeneracy of the energy levels, the rate of spontaneous emission, and the energy of the emitted photon; ΔE is the energy gap between the two excited levels; k is the Boltzmann constant; and T is the absolute temperature. The relative change in the temperature is determined from the intensity ratio for the characteristic peaks of the luminescence of up-conversion particles as

$$\ln[I_1(\lambda_1)/I_2(\lambda_2)] = \ln C - \Delta E/kT. \quad (2)$$

3. Results and discussion

Figure 4 presents the images of decapods C. multidentana before and after administration of up-conversion particles. One can see that under irradiation of decapods with the laser light at the wavelength 975 nm, the luminescence signal of up-conversion particles appears to be so strong that it is seen with naked eye and, in contrast to the methods of confocal fluorescence microscopy [17, 18], does not require special conditions for conducting the experiment.

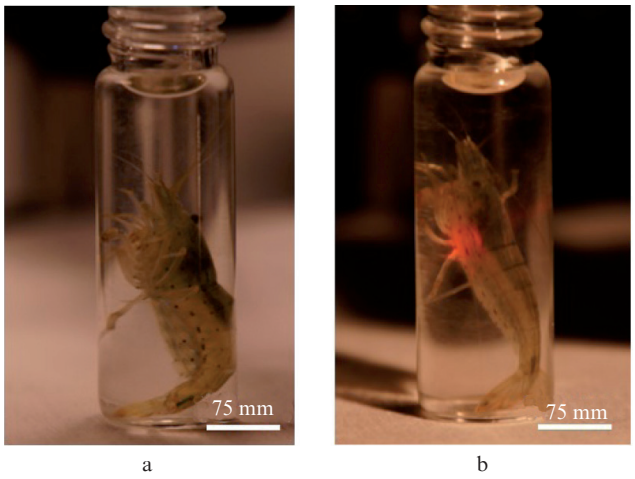

Figure 4. (Colour online) Images of C. multidentata decapods irradiated by the laser at the wavelength 975 nm (a) before and (b) after the injection of luminescent particles.

Using the data presented in Fig. 5 and Eqn (2), one can determine the energy gap between two excited states ΔE. In our case $\Delta E = 2.6\times10^{-18}$ J (16.23 eV) for the particles in aqueous medium and 4.99×10^{-18} J (31.19 eV) for the biological object. The observed temperature-dependent difference of the luminescence spectra of up-conversion particles is due to the composition of the studied media, the deionised water and haemolymph of decapods, containing a wide spectrum of ions (mainly, Na^+ and Cl^-), a number of proteins (mainly the breathing pigment haemocyanin) and the cell components.

Thus, it is shown that the temperature gradient in the aquatic organisms *in vivo* can be determined using the up-conversion particles injected in a biological object. Since the temperature is one of the crucial parameters for poikilothermic species, the temperature measurements of inner tissues of aquatic organisms largely allows the characterisation of the biological object condition in real time. The difference between the temperature of the internal tissues of the organism and the temperature of environment is an indicator of metabolic activity of individual organs and the mobility activity of the entire organism.

It is worth noting that the presented method has a number of essential advantages as compared to the alternative optical

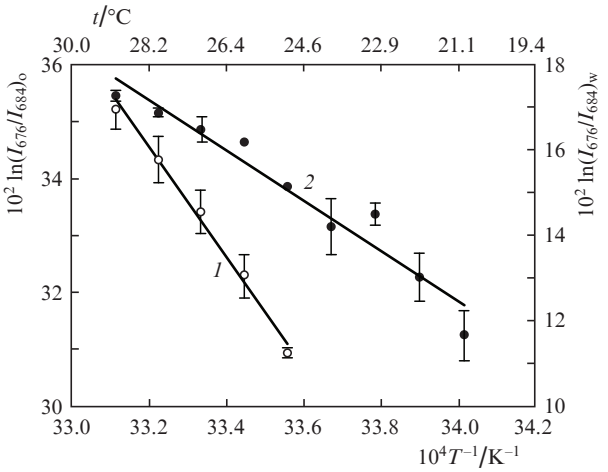

Figure 5. Temperature dependence of the mean value of the intensity ratio for the fluorescence of up-conversion particles. The environment temperature was changed from 25 °C to 30 °C with a step 1 °C. The intensity ratio was calculated for the wavelengths 676 nm and 684 nm for the particles, injected into the biological object [(*1*), left-hand scale] and the particles in aqueous medium [(*2*), right-hand scale]. The points are experimental values, and the straight lines are approximations.

methods of estimating the stress conditions in aquatic organisms [9, 17, 18]. In particular, the use of up-conversion particles, as already mentioned, does not require special conditions for performing the experiment, in contrast to the methods of confocal fluorescence microscopy [17, 18]. The specific position of the laser probing wavelength in the IR region and the intensity maxima in the luminescence spectrum of the detected signal allows the elimination of the background signal of the biotissue, in contrast to the methods of optical spectroscopy [9], where the background radiation is parasitic [19]. Besides that, although the luminophores have a high photochemical stability comparable to that of quantum dots, they are essentially less toxic [20–22] and possess stable narrowband emission under excitation in the near IR region [23]. As compared to the microspheres and microcapsules containing fluorescent dyes [17, 18] or quantum dots [23], the luminophores allow the observation of the signal in the absence of biotissue autofluorescence, which significantly increases the signal-to-noise ratio in practice. In the experiments presented in this paper, the signal-to-noise ratio amounted to 13.4 (λ = 561 nm, 25 °C) and 287 (λ = 658 nm, 25 °C) for the control sample (aqueous suspension of the particles), and 11.6 (λ = 561 nm, 25 °C) and 260 (λ = 658 nm, 25 °C) for the particles administered into the biological object. The fluorescence lifetime of organic fluorophores lies in the nanosecond range, while the lifetime of luminescence in the particles used by us is on the order of microseconds [24]. Thus, the difference in lifetime allows one to eliminate the signal of parasitic background luminescence not only in the frequency domain, but also in the time domain, which increases the signal-to-noise ratio even more.

4. Conclusions

Thus, by the methods of ecophotonics we have studied the possibility of using the luminescence spectroscopy in combination with up-conversion particles for monitoring the condition of aquatic organisms aimed at the investigation and prognosis of the consequences of negative effects of human activity and climatic changes. The possibility of a noninvasive measurement of temperature inside the decapods *in vivo* is demonstrated in principle, using the thermosensitive luminescent up-conversion particles Y_2O_3:Yb,Er, injected into the biological object (Caridina multidentata). The linear character of the temperature dependence of the logarithm of intensity ratio of peaks in the luminescence spectrum of up-conversion particles is found. This fact allows the use of the proposed method for quantitative assessment of metabolic and motional activity of aquatic organisms and, generally, opens the prospects of developing the luminescence spectroscopy methods in combination with up-conversion particles in ecological applications.

Further development of the technique proposed in the present paper, in analogy with Refs [25, 26], implies the testing of luminescent up-conversion particles as a relatively cheap sensor for noninvasive express estimate of such parameters, as the oxygen and the carbon dioxide content, the pH value, and the presence of certain bacteria and/or toxic biochemical compounds. The new line of research, the ecophotonics, is expected to join different branches of ecology, ecophysiology, photonics, biology, physiology, and biochemistry and will facilitate the development of most promising studies aimed at monitoring the condition of aquatic organisms, the changes of which can be related to the negative consequences of climatic changes, enhanced by the human activity.

Acknowledgements. The work was partially supported by the Academy of Finland (Project No. 288164, support of E.V. and I.Ya., Projects Nos 260321, 290596, support of A.P. and A.B.), the Government of Russian Federation (Grant No. 074-U01), and the Russian Science Foundation (Grant No. 15-14-10008) (support of A.G., E.B., M.T., and I.M.)

References

1. Parmesan C. *Annu. Rev. Ecol. Evol. Syst.*, **37**, 637 (2006).
2. Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., Miller H.L. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge: Cambridge University Press, 2007).
3. Lannig G., Cherkasov A.S., Portner H.O., Bock C., Sokolova I.M. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **294**, R1338 (2008).
4. Scheffer M., Carpenter S., Foley J.A., Folke C., Walker B. *Nature*, **413**, 591 (2001).
5. Maler K.-G. *Eur. Econ. Rev.*, **44**, 645 (2000).
6. Hoffmann A., Hercus M. *Bioscience*, **50**, 217 (2000).
7. Teotonio H., Rose M. *Nature*, **408**, 463 (2000).
8. Badyaev A. *Proc. Roy. Soc. B*, **272**, 877 (2005).
9. Axenov-Gribanov D.V., Gurkov A.N., Shakhtanova N.S., Bedulina D.S., Timofeyev M.A., Meglinski I. *J. Biophotonics*, **4** (9), 619 (2011).
10. Meglinski I.V., Kuz'min V.L., Priezzhev A.V. *Quantum Electron.*, **36**, 989 (2006) [*Kvantovaya Elektron.*, **36**, 989 (2006)].
11. Tuchin V.V. *Opticheskaya biomeditsinskaya diagnostika* (Optical Biomedical Diagnostics) (Moscow: Fizmatlit, 2007).
12. Tuchin V.V. *Handbook of Optical Biomedical Diagnostics* (Bellingham, WA: SPIE Optical Engineering Press, 2002).
13. Lakowicz J.R. *Principles of Fluorescence Spectroscopy* (Springer, 2006).
14. Auzel F. *Chem. Rev.*, **104** (1), 139 (2004).
15. Kuznetsova Yu.O. *Izv. Samarskogo Nauchn. Tsentra RAN*, **15** (4), 113 (2013).
16. Maestro L.M., Rodriguez E.M., Rodríguez F.S., Iglesias-de la Cruz M.C., Juarranz A., Naccache R., Vetrone F., Jaque D., Capobianco J.A., Sole J.G. *Nano Lett.*, **10**, 5109 (2010).

17. Sadovoy A., Teh C., Escobar M.V., Corzh V., Meglinski I. *Laser Phys. Lett*., **6**, 542 (2012).
18. Sadovoy A., Teh C., in *Biophotonics for Medical Applications*. Ed. by I.Meglinski (Cambridge: Elsevier, 2014) Woodhead Publishing Series in Biomaterials, Ch. 11, pp 321–330.
19. Tuchin V.V. *Lazery i volokonnaya optika v biomeditsinskikh issledovaniyakh* (Lasers and Fibre Optics in Biomedical Studies) (Moscow: Fizmatlit, 2010).
20. Lin C., Berry M.T., Anderson R., Smith S., May P.S. *Chem. Mater.*, **21**, 3406 (2009).
21. Guller A.E., Generalova A.N., Petersen E.V., Nechaev A.V., Trusova I.A., Landyshev N.N., Nadort A., Grebenik E.A., Deyev S.M., Shekhter A.B., Zvyagin A.V. *Nano Res*., **8** (5), 1546 (2015).
22. Popov A.P., Karmenyan A.V., Bykov A.V., Khaydukov E.V., Nechaev A.V., Bibikova O.A., Panchenko V.Y., Semchishen V.A., Seminogov V.N., Akhmanov A.S., Sokolov V.I., Kinnunen M.T., Tuchin V.V., Zvyagin A.V. *Proc. SPIE Int. Soc. Opt. Eng*., **8801**, 88010C (2013).
23. Chatterjee D.K., Yong Z. *Nanomed. J*., **3**, 73 (2008).
24. Zherdeva V.V., Savitskii A.P. *Usp. Biol. Khim*., **52**, 315 (2012).
25. Moczko E., Meglinski I., Bessant C., Piletsky S.A. *Anal. Chem*., **81**, 2311 (2009).
26. Moczko E., Chauchi M., Turner C., Meglinski I., Piletsky S.A. *IEEE Trans. Bio-Med. Eng*., **58**, 2154 (2011).