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# Effect of light scattering on biological tissue thermometry from photoluminescence spectra of up-conversion nanoparticles

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*Abstract.* **Photoluminescence spectra of the up-conversion nanoparticles $NaYF_4:Y^{3+}$, $Er^{3+}$ arranged under a layer of an adipose tissue have been recorded in a wide temperature range. It is shown that if nanoparticle temperature is determined from the photoluminescence spectra, an error arises related to spectrum distortion due to light scattering in a sample. A correction algorithm for reducing this error is developed.**

***Keywords:*** *up-conversion nanoparticles, adipose tissue, photoluminescence spectrum, optical clearing, laser, thermometry.*

## 1. Introduction

The employment of nanoparticles (NPs) as a thermometer for precise remote temperature measurements with real-time micron spatial resolution is important in many practical applications for laser medicine and biology [1–3].

Modern fluorescent nano-thermometers allow sufficiently accurate (to tenth of degree) measurements of a local temperature in a thin layer of a biological object [4]. For this purpose, temperature-dependent variations of NP luminescence characteristics are used. Such characteristics may be the variation of photoluminescence intensity due to temperature quenching [4–6] and a shift of the spectral band, because for a number of NPs the position and value of the fluorescence spectrum maximum depend on ambient temperature [5, 7].

However, the detection of photoluminescence of NPs localised inside biological tissues is a complicated task [8]. While using luminescent NPs for solving biological problems, one should take into account the fact that the obtained result is affected by the interaction of radiation with a biological object. The effects of multiple light scattering and re-absorption in biological tissues distort excitation, photoluminescence, and absorption spectra of NPs.

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Absorption of photoluminescence inside a sample depends on both the absorption coefficient and the degree of photoluminescence scattering in the medium at a particular wavelength. The parameters mentioned depend on the wavelength; hence, the degree of light attenuation varies in a sufficiently wide spectral range both for photoluminescence and for exciting radiation.

As a thermometer, one can use up-conversion nanoparticles (UCNPs) capable of absorbing several photons in the near-IR spectral range with the following emission in the visible range. The employment of up-conversion has some advantages as compared to conventional luminescence. Those are a large penetration depth of the laser radiation that excites NP photoluminescence and actually totally absent intrinsic photoluminescence of a biological tissue in the spectral range of UCNP emission, which gives a chance to exclude the tissue background signal [9–13]. Temperature measurements by UCNP are performed by recording the intensity ratio for two close bands of photoluminescence. Since a photoluminescence intensity is proportional to the population of the excited level, the ratio of intensities for two close bands is described by the Boltzmann law:

$$I_1(\lambda_1)/I_2(\lambda_2) = C\exp[-\Delta E/(k_B T)], \quad (1)$$

where $I_1(\lambda_1)$ and $I_2(\lambda_2)$ are the radiation intensities for two transitions; $C$ is the normalised constant dependent on the degree of energy level degeneration, probability of spontaneous emission, and energy of emitted photons; $\Delta E$ is the energy difference for excited states; $k_B$ is the Boltzmann factor; and $T$ is the absolute temperature.

The possibility of using nano-thermometers, for example, $NaYF_4:Er^{3+},Yb^{3+}$ particles, is determined by a sensitivity to temperature of fluorescence intensities of the $Er^{3+}$ ion bands in the green range, corresponding to transitions $^4S_{3/2} \rightarrow {}^4I_{15/2}$ ($\lambda_1 = 546$ nm) and $^4H_{11/2} \rightarrow {}^4I_{15/2}$ ($\lambda_2 = 520$ nm). The excitation of the $^2F_{5/2}$ level of the $Yb^{3+}$ ion and the following energy transfer from the $Yb^{3+}$ ion to $Er^{3+}$ lead to acceptor transfer to the $^4F_{7/2}$ level with the following transfer to the $^4S_{3/2}$ level in the result of nonradiative relaxation (Fig. 1). While the thermal equilibrium is being established, the $^2H_{11/2}$ level is also populated. The $Er^{3+}$ ion can transfer to the fundamental state from levels $^4S_{3/2}$ and $^2H_{11/2}$ emitting in the green spectral range at wavelengths of ~550 and ~525 nm, respectively. Each of the bands comprises several narrow emission peaks related to the Stark splitting of the low level [4].

We have calculated natural logarithm values for the ratio of NP photoluminescence intensities at $\lambda_1$ = 546 nm

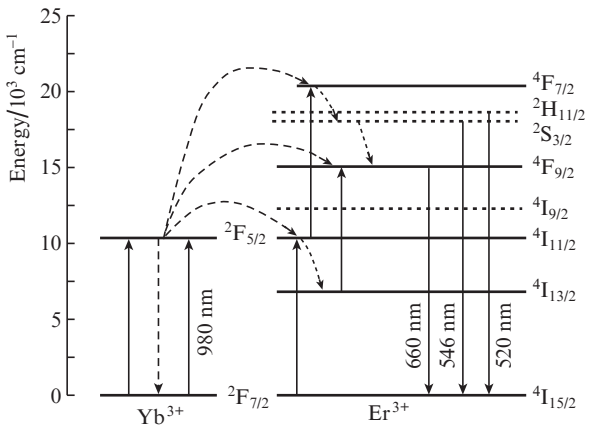


**Figure 1.** Energy level diagram in a system of $Yb^{3+}$ and $Er^{3+}$ ions.

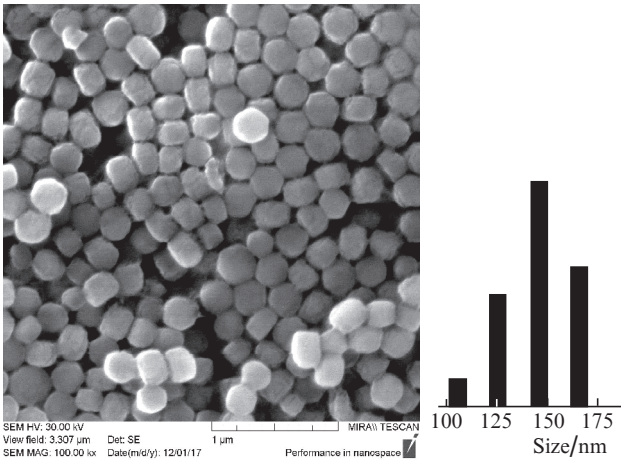


**Figure 2.** (a) Image of $NaYF_4:Er^{3+}$, $Yb^{3+}$ UCNPs obtained with a MIRA 2 LMU scanning electron microscope (TESCAN), and (b) NP size distribution.

and $\lambda_2 = 520$ nm; the corresponding temperature dependence is approximated as follows

$$\ln(I_2/I_1) = -\ln C + \Delta E/(k_B T) = A + B/T. \quad (2)$$

According to the calculations, the inaccuracy of luminescence intensity measurement of 1% yields an error of 0.6% in temperature measurements. The inaccuracy of 1% in determining the slope of the $B$ dependence yields the error of 3 °C. In turn, a variation of the $A$ parameter by 1% results in an error of 2 °C.

The coefficient $A$ depends on experimental conditions. In particular, detected luminescence intensities differ from nanoparticle luminescence intensities $I_1$ and $I_2$ due to attenuation in the result of scattering and absorption inside a sample.

The present work is aimed at studying an influence of light scattering and absorption in a sample on the accuracy of temperature measurements by NPs arranged in a biological tissue.

## 2. Materials and methods

We have synthesised thermosensitive $NaYF_4:Yb^{3+}$, $Er^{3+}$ UCNPs, placed them into a cellulose acetate film, and used for measuring a local temperature of a biological tissue. NPs were synthesised by the hydrothermal method and covered by a $SiO_2$ shell [14]. An average particle size was 150 nm (Fig. 2). For producing the film, 1 g of cellulose triacetate was dissolved in 1 g of acetone. An NP powder (1 mg) was added to the solution. After mixing and homogenising by ultrasound the solution was poured on a glass surface and dried slowly. The film obtained had the NP concentration of 0.1 mass percent and thickness of 150 µm.

The setup used in experiments is schematically shown in Fig. 3. UCNP photoluminescence was excited by radiation of an LSR980NL-1000 laser ($\lambda$ = 980 nm, 108 mW) (Lasever, China). Photoluminescence spectra of the UCNPs arranged under a layer of a biological tissue sample were recorded by using a QE6500 FL fibre-optic spectrometer (Ocean Optics, USA) with a fibre probe that was perpendicular to the sample surface at a distance of 25 mm from it. The exciting beam diameter was 2.3 mm. A diameter of the probe fibre core was 400 µm, and the fibre aperture was 0.22. In front of the fibre-optical probe, there was a collimating lens of diameter 25 mm with a focal distance of 10 mm. The end of the fibre probe was in a focus of the lens. In view of the small thickness of used biological samples, one can believe that the detection scheme collected collimated radiation from a sample surface area of the diameter 4.5 mm, which is twice a dimension of the exciting spot. Scattered exciting radiation is suppressed by placing a SZS21 filter (State Standard GOST 9411-91) in front of the spectrometer, which provides a transmission coefficient of $10^{-6}$ at the excitation wavelength and 0.96 in the range of luminescence recording at the filter thickness of 5 mm. The accuracy of luminescence intensity measurements was 0.5%.

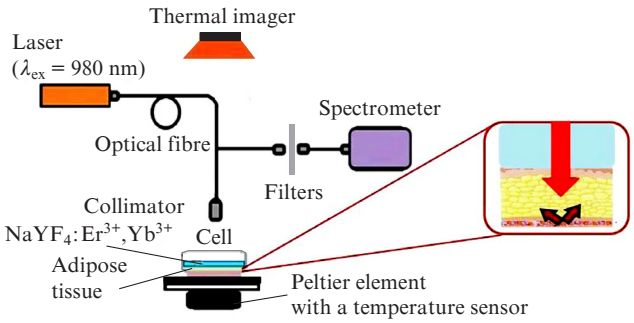


**Figure 3.** Schematic of the experimental setup.

Spectra of tissue sample collimated transmission were recorded by using a USB4000-Vis-NIR multichannel spectrometer (Ocean Optics, USA). The samples mounted on a glass were arranged between two QP400-1-VIS-NIR optical fibres (Ocean Optics, USA) with the internal diameters of 400 µm. 74-ACR collimators (Ocean Optics, USA) collimated the radiation. The radiation source was a HL-2000 halogen lamp (Ocean Optics, USA). A measurement error was at most 5%. Sample thicknesses were measured before and after the spectral recording. The temperature was varied by heating the glass at an accuracy of 1 °C.

The biological objects were samples of abdominal adipose tissue *in vitro* (10 samples) taken in the process of plastic surgery (five men 40–50 years old with a weight of 70–80 kg). Thin tissue slices (0.1–0.4 mm) obtained from frozen samples

were used for investigations. Experiments were conducted at the Centre for Joint Use of Saratov State Medical University (Russia).

A sample of adipose tissue was placed inside a glass cell with the cell bottom thickness of 0.5 mm. A film with UCNPs was glued to the bottom side of the cell. An adipose tissue sample was heated through the cell bottom by a Peltier element equipped with a temperature control PTP1 (Perkin Elmer, USA). A temperature of the upper surface of investigated objects was measured remotely by a thermal-infrared camera (thermal imager) IRISYS IRI 4010 (120 × 160 pixel) (Great Britain).

Due to a good thermal contact, the temperature of NPs corresponded to that prescribed by the Peltier element. The NP temperature varied from 20 to 60 °C with a step of 5 °C. The adipose tissue temperature measured by the thermal imager differed from NP temperature by 1–3 °C.

## 3. Results and discussion

In Fig. 4, one can see temperature dependences of logarithm of the photoluminescence intensity ratio for a UCNP polymer film at the wavelengths $\lambda_1 = 546$ nm and $\lambda_2 = 520$ nm and for the same UCNP samples covered with a 0.1-mm layer of adipose tissue. One can see that the dependences are shifted with respect to each other.

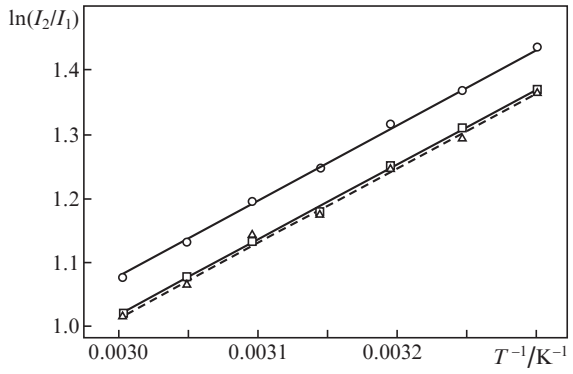


**Figure 4.** Temperature dependences $\ln[I_2(\lambda_2)/I_1(\lambda_1)]$ at $\lambda_1 = 546$ nm and $\lambda_2 = 520$ nm for a UCNP polymer film (□) and similar film with an adipose tissue layer of thickness 0.1 mm (○), and the dependence for the UCNP film with the adipose tissue corrected for photoluminescence absorption and scattering in the sample (△).

Since the slope of a straight line in linear approximation (2) is determined by the energy difference for the excited erbium levels, it can be considered constant for both the experimental dependences obtained. Approximation in the case of the clean film yields the following coefficients: $A = 2.488 \pm 0.001$ and $B = 1169 \pm 0{,}5$. For the film with adipose tissue we obtained $A = 2.427 \pm 0.002$, which is explained by a reduction of the recorded luminescence intensity after passing through the adipose tissue.

In order to develop a correction algorithm we obtained experimental data on the variation of the transmission coefficient of adipose tissue under varied temperature (Fig. 5). Then, we used the model of the collimated radiation recording for correction. In that case, the intensity of recorded radiation is described by the formula

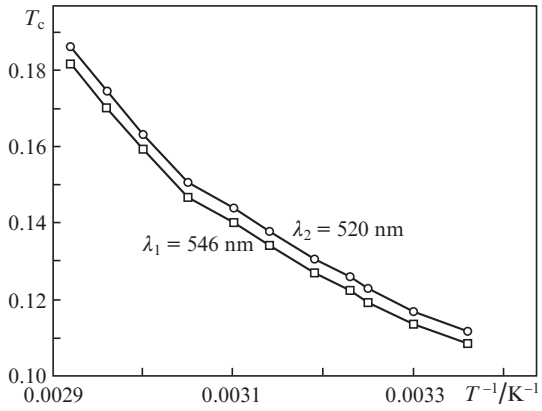


**Figure 5.** Temperature dependences of the collimated transmission coefficient $T_c$ for an adipose tissue sample at the radiation wavelengths $\lambda_1$ and $\lambda_2$.

$$I_{reg} = I_{lum} T_c, \tag{3}$$

where $T_c$ is the collimated transmission coefficient at the wavelength of luminescence recording.

Thus, the ratio of photoluminescence intensities in (2) transforms to the form

$$\ln \frac{I_{reg2}}{I_{reg1}} = \ln \frac{I_{lum2}}{I_{lum1}} + \ln \frac{T_{c_2}}{T_{c_1}} = A + \frac{B}{T} + \ln \frac{T_{c_2}}{T_{c_1}}. \tag{4}$$

A result of the correction is presented in Fig. 4. One can see that, except for the deviations that may result from the detection inaccuracy of the photoluminescence attenuated in a sample, the corrected curve actually coincides with that for the film with NPs. That is, taking into account photoluminescence attenuation in the sample gives a chance to find a real temperature value for NPs imbedded into the sample. The value of parameter $A + \ln(T_{c_2}/T_{c_1})$ calculated in this approximation is $2.493 \pm 0.003$, which is substantially closer to the value of $A$ obtained in the case of the film without adipose tissue. An estimated error of temperature measurements is 5 °C without correction and 0.5 °C with data correction.

Thus, we have shown that absorption and scattering of NP photoluminescence in a sample distorts the NP temperature data obtained from their luminescence characteristics. The distortion arises even in a case of a weakly absorbing and scattering adipose tissue of thickness ~0.1 mm. The algorithm developed for data correction is capable of obtaining a real temperature of NPs.

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