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# Changes in the spectral characteristics of biological tissues depending on temperature

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## ABSTRACT

This work was aimed at determining the optical characteristics of biological samples at different temperatures. The work investigated the change in the intensity and shape of the absorption spectra of various biological tissues in vitro, depending on the sample temperature.

**Keywords:** spectral characteristics, temperature, biological tissues, collimated transmission spectra

## 1. INTRODUCTION

Internal temperature of organs and tissues is an important parameter for monitoring physiological processes [1] in the body. Based on information about the distribution of internal temperature, conclusions about the state and functioning of organs [2], and the reaction of the human body to external influences can be drawn. In particular, methods of diagnosing diseases of internal organs can be based on the measurement of the internal temperature, and monitoring of inflammatory processes can be carried out. Nano-thermometry allows high-resolution measurements of intracellular temperature. This makes it a promising tool for the study of cell physiology, since a detailed measurement of the temperature distribution can reveal the characteristics of cell metabolism and its response to various external stimuli [3, 4]. However, such measurements cannot be performed non-invasively for diagnostic purposes.

Upconversion nanoparticles like  $\text{NaYF}_4: \text{Er}^{3+}, \text{Yb}^{3+}$  are one of the types of nanothermometers [5]. The sample temperature is determined from the spectrum of upconversion luminescence of the nanoparticles using the ratio of the intensities of the 510-530 and 535-560 nm bands (Fig. 1).

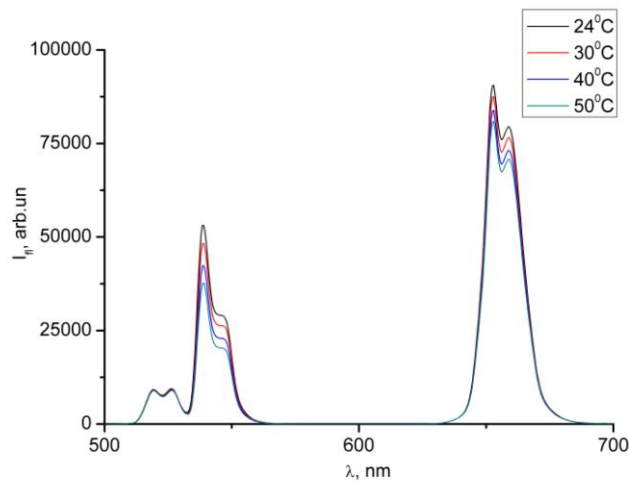


Figure 1. Luminescence spectra of  $\text{NaYF}_4: \text{Er}^{3+}, \text{Yb}^{3+}$  upconversion nanoparticles at different temperatures (excitation at a wavelength of 980 nm).

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The scattering and absorption parameters of biological tissues through which the luminescence passes on its way to the detector depend on the wavelength. As a consequence, luminescence of different wavelengths is attenuated differently, which in turn distorts the intensity ratio. To reliably estimate the sample temperature, the luminescence attenuation must be estimated accurately enough. In so doing, a change in the optical properties of biological tissues caused by a change in temperature should be taken into account. In addition, a high temperature of biological tissue generated during therapy can cause coagulation of healthy tissue surrounding the affected area. In this case, light scattering increases sharply. Changes in spectra caused by scattering can be a tool for monitoring coagulation processes. In this paper, we report the optical characteristics of biological samples at different temperatures.

## 2. RESULTS AND DISCUSSION

Previously, we studied the spectra of rat skin [6], muscle and adipose tissues using an integrating sphere. We characterized the changes in the spectral characteristics of rat skin as a result of skin heat treatment at elevated temperatures. To detect temperature changes, the tissues were heated at a given temperature, after which the spectra of total transmission and diffuse reflection were recorded sequentially. On their basis, the absorption and scattering factors of the biological objects were calculated. It was shown that the dynamics of the changes in collimated transmission of rat skin depends on temperature. In this case, changes occur quickly, within 3-5 minutes. Subsequent changes are small. The most dramatic changes occur at temperatures above 45° C. It is assumed that changes in spectral characteristics are caused by changes in light scattering in the sample. An increase in the size of the scatterers upon heating the samples was shown. It was also concluded that the refractive index of collagen fibers changes upon heating.

However, the design of currently produced spectrophotometers does not allow simultaneous registration of both spectra. At the same time, registration in one mode, for example, of diffuse reflection, with subsequent averaging is quite meaningless. The passage of light through a scattering medium is described by the transport equation and does not obey Bouguer's law. The transmission and reflection spectra of these objects depend on both their optical parameters and their thickness. The thickness of object slices inevitably varies, in accordance with which the spectra also change. Due to the nonlinear nature of the dependences, the averaged spectrum for the averaged sample thickness does not provide useful information.

Previously, we also recorded the transmission and reflection spectra of the samples using the following technique: the samples were heated to a given temperature, held for some time [7]. Then the samples were cooled to room temperature and the transmission and reflection spectra were sequentially recorded. Temperature dependences of the spectral characteristics of the rat skin, muscle and fat tissues were obtained in vitro. The changes in the spectral characteristics of the rat skin subjected to 60 min thermal treatment at elevated (>40°C) temperatures have been characterized. The dynamics of the changes in collimated transmittance of rat skin at elevated temperature is shown to be temperature-dependent. The heating of rat skin samples leads to a change in the anisotropy factor due to coagulation of scatterers. The change in skin absorption induced by heating begins at temperatures of 60-70 °C due to the greater temperature stability of the skin compared to muscle tissue.

The absorption spectrum of mainly hemoglobin is superimposed on the scattering spectrum of muscle tissue. Heating changes the structure of hemoglobin, which affects the dynamics of the light absorption. Tissue coagulation is absent while heating muscle tissue samples to a predetermined temperature at temperatures up to 60 °C. At 70 °C, heating leads to increased absorption.

Phase transitions occur already in the process of heating adipose tissue to a predetermined temperature. The degree of coagulation and, accordingly, the absorption coefficient depend on the heating rate and temperature. Namely, the higher the temperature rises, the greater the initial value of the absorption coefficient we have.

This technique provides information on the changes arising from the heating of the sample. However, it is impossible to obtain spectral data on biological tissues directly at a given temperature - when the sample is cooled, its characteristics change.

In this regard, studies of the characteristics of biological tissues were aimed at studying collimated transmission directly at a given temperature and the attenuation of collimated radiation calculated from it. Such studies make it possible to

obtain correct statistics for the temperature changes of the measured signal and, in addition, data on collimated transmission are used in the developed technique for correcting the luminescence spectra.

For these measurements, a setup was used, in which the entire optical path, starting from the collimators, was placed in a heating cabinet with a temperature controlled with an accuracy of 0.1°C (Figure 2).

Tissue samples of healthy rats were examined: skin with hair and fat removed, muscle tissue, subcutaneous adipose tissue. To prevent distortions caused by reflections on a glass slide, the samples were fixed on a frame and placed into a heating cabinet heated to 35.5 °C to store all samples at a given temperature. The cabinet was kept at a saturated humidity to prevent drying of the samples. A series of spectra was recorded for each sample at an initial temperature (35.5°C).

Then, with a heating step of 5°C, the next series of spectra was recorded. During the experiment, the samples were not removed from the cabinet and were heated gradually (the heating cabinet has a heating rate of about 1°C for 5 minutes). The spectra were recorded in the temperature range 35.5-45°C, since at 50°C coagulation of tissue proteins begins. Moreover, at 50°C, despite the maintaining of humidity inside the cabinet, the samples begin to lose water. This reduces the thickness and weight, and increases the transparency of the samples.

Figures 3-6 show the characteristic spectra of the attenuation coefficients of collimated radiation of the samples. It was revealed that for the skin, as a absorbing and scattering multilayer medium, the attenuation coefficients for radiation passing from the outside (from the epidermis) and from the inside (from the dermis) are different. When light passes from the side of the dermis, the attenuation of light is less, which may be due to the fact that the scattering of light in the epidermis is higher than in the dermis. Accordingly, this fact must be taken into account when calculating the luminescence attenuation coefficients and spectra correction.

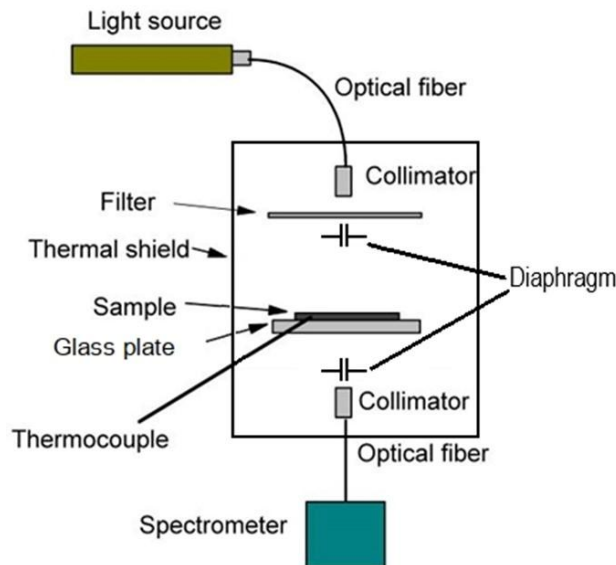


Figure 2. Block diagram of a setup for recording collimated transmission of biological tissues at different temperatures

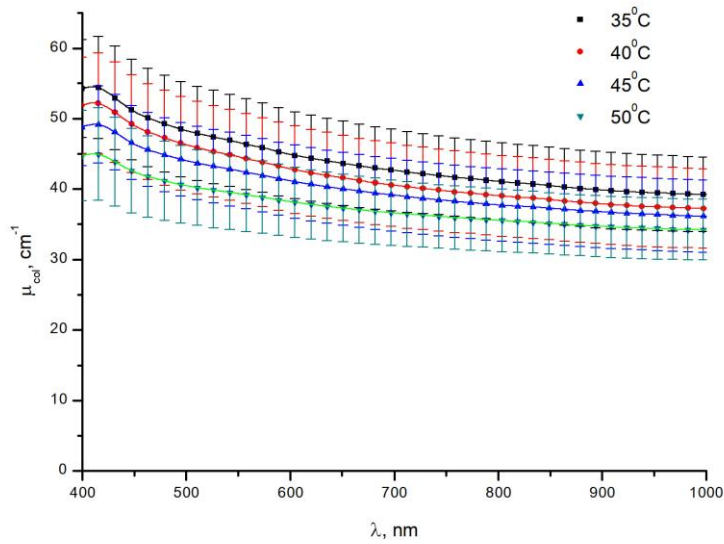


Figure 3. Spectra of collimated light transmission of rat skin when light passes from the epidermis.

It should be noted that these results differ from those obtained earlier, performed without maintaining humidity. Perhaps this is due to the drying of the upper and lower layers of the sample, as a result of which the reflection and scattering of light on the layers increase.

Figure 5 shows the spectra of rat muscle tissue, obtained at different temperatures. The resulting changes can be associated with phase transitions in adipose tissue, which is inevitably present in the samples. These changes are consistent with the behavior of the spectra of adipose tissue at different temperatures (Figure 6).

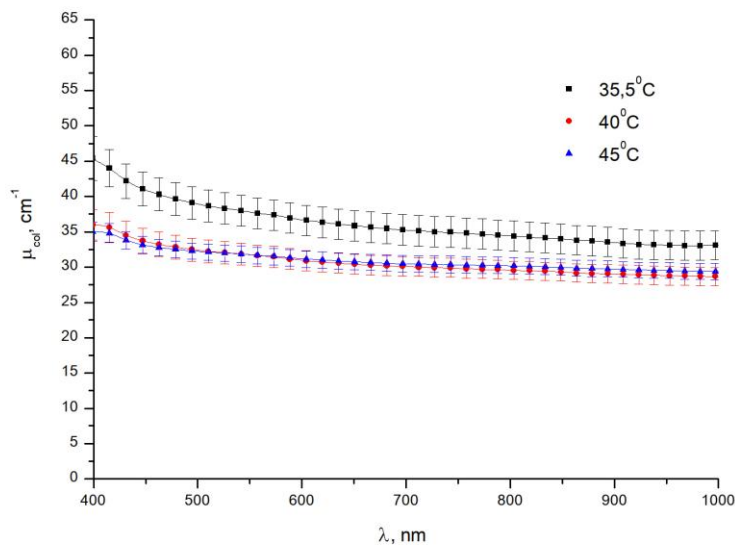


Figure 4. Spectra of collimated light transmission of rat skin when light passes from the dermis.

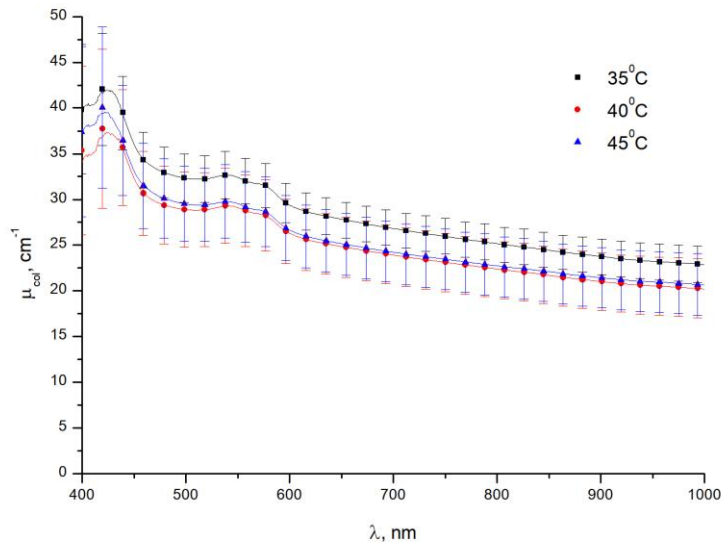


Figure 5. Collimated light transmission spectra of rat muscle tissue.

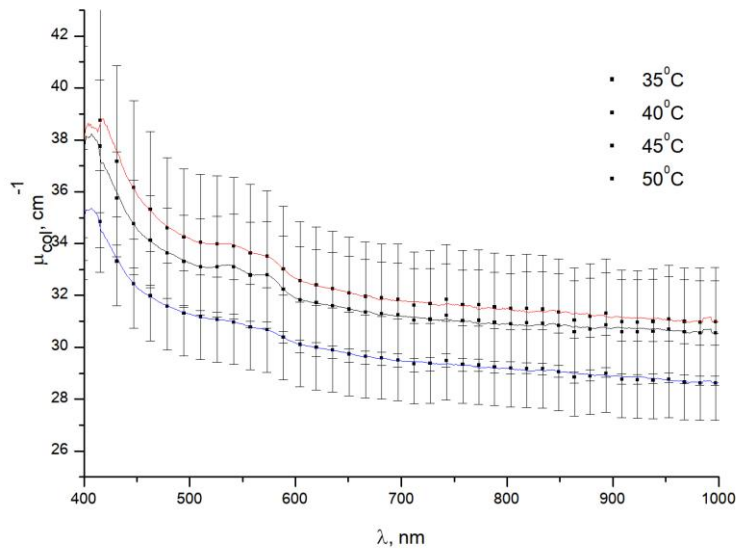


Figure 6. Collimated light transmission spectra of rat adipose tissue.

### 3. CONCLUSIONS

The studies of collimated transmission spectra of rat skin, muscle and subcutaneous adipose tissue samples were carried out under conditions of temperature stabilization in the range of 35-50°C, as well as environmental stabilization (at saturated water vapor pressure). It has been shown that maintaining the humidity makes it possible to exclude spectral distortions due to drying and increased tissue coagulation at temperatures up to 45°C. At high temperatures, the state of the surface layers of the sample changes, which leads to an increase in scattering in it. The implemented conditions make it possible to obtain results that are closest to those expected when exposed to heat or laser heating in vivo.

## ACKNOWLEDGMENTS

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