1550nm Infrared Laser Heating NaYF₄:Er³⁺ Nanoparticle In Isolated Pig Liver and Up-conversion Fluorescence Intensity Ratio Temperature Measurement

Xinyu He ¹, Songsong Liao ¹, Zhuohong Feng ², Wenqi Huang ² and Yantang Huang ¹,*

¹ College of Physics and Information Engineering, Fuzhou University, Fuzhou 350116, China
² College of Physics and Energy, Fujian Normal University, Fuzhou 350117, China
* huangyantang@fzu.edu.cn

Abstract. Hyperthermia is an important means of cancer treatment which is called "tumor green therapy". The clinical heat therapy heating sources include ultrasound, microwave, alternating magnetic field, infrared laser. However, it is still difficult to accurately measure the temperature of tumor hyperthermia and the dose of hyperthermia. Here, NaYF₄:Er³⁺ nanoparticles were injected into a small part of isolated pig liver, and heated it with 1550nm infrared laser. At the same time, the laser excited the NaYF₄:Er³⁺ nanoparticles to generate up-conversion green fluorescence. The fluorescence intensity ratio was used to measure the temperature of the heating part in real time.

1. Introduction
Cancer is one of the leading diseases facing mankind. At present, the treatment of tumor mainly includes surgical therapy, chemotherapy, radiotherapy, and immunotherapy. Hyperthermia (HT) has become the fifth therapy after them [1]. The treatment can be applied in combination with other verified cancer treatments using several different procedures. It is a new and effective method with less toxic and side effects in the treatment of tumor, which is called "tumor green therapy". Hyperthermia will not cause adverse reactions such as bone marrow suppression and hair loss, nor will it cause any pollution to people and the environment. The principle is that most tumor cells can be induced into apoptosis at 42.5 to 43.5°C for 60 to 120 minutes, but human normal cells can tolerate the temperature for a long periods of time without damage (Indeed, healthy tissues are able to withstand temperatures of 42-45 °C, in contrast to cancer cells which undergo apoptosis at those temperatures, The temperature safety limit of normal tissue cells is 45±1°C) [2-3]. Because of the distortion and expansion of blood vessels in tumor tissues, that results in high blood flow resistance, imperfect vascular receptors, and poor sensitivity to temperature. It is difficulty in heat dissipation under high temperature, so the tumor is rapid warming, forming a huge heat reservoir, and can form a temperature difference of 5-10°C with normal tissues. The clinical heat therapy heating sources include ultrasound, microwave, alternating magnetic field, infrared laser. However, it is still difficult to accurately measure the temperature of tumor hyperthermia and the dose of hyperthermia [4].

In this paper, NaYF₄:Er³⁺ nanoparticles were injected into a small part of isolated pig liver, and heated it with 1550nm infrared laser. At the same time, the laser excited the NaYF₄:Er³⁺ nanoparticles to generate up-conversion green fluorescence. The fluorescence intensity ratio was used to measure
the temperature of the heating part in real time. The experiment paves the way for further research into the killing of cancer cells by live-body heat therapy in animals.

2. Principle of FIR Temperature Measurement
Among the main optical temperature sensing technologies, the FIR technology based on the thermal coupling energy level of rare earth ions is less dependent on the measurement conditions [5]. Therefore, FIR can effectively reduce the measurement errors caused by non-temperature parameters. The relative intensity ratio of the two emission peaks from the thermal coupling energy level changes when the temperature increases. Therefore, real-time temperature detection can be achieved by recording the FIR value.

Based on the principle of thermodynamic statistics, when two energy levels are close to the level difference of 200–2000 cm$^{-1}$, the relaxation time for the system to reach thermal equilibrium is significantly shorter than the fluorescence lifetime, and the number of particles on the two energy levels can quickly reach the thermal equilibrium. Therefore, the pair of energy levels is called “thermally coupled energy levels” [6-8]. As we know, the absorption spectrum of the $^2\text{H}_{11/2}$ and $^2\text{S}_{3/2}$ energy levels of the Er$^{3+}$ ions is approximately 712 cm$^{-1}$, which makes them suitable for this application. Therefore, we select NaYF$_4$:Er$^{3+}$ phosphor based on the FIR technology to develop temperature sensors.

Figure 1 depicts a simplified energy level structure diagram of rare earth ions. In this figure, energy levels $L_1$ and $L_2$ form a pair of thermally coupled energy levels. The ratio of the numbers of electrons ($N_1$ and $N_2$) occupying the two energy levels under excitation satisfy the Boltzmann distribution:

$$\frac{N_2}{N_1} = \frac{g_2}{g_1} \exp\left(-\frac{\Delta E_{21}}{k_B T}\right)$$

(1)

where $g_1$ and $g_2$ are the degeneracies of $L_1$ and $L_2$, respectively; $\Delta E_{21}$ is the energy gap of the thermally coupled energy level; $k_B$ is the Boltzmann constant; and $T$ is the absolute temperature.

In figure 1, the fluorescence intensity of the transition radiation from $L_1$ and $L_2$ in the excited state to $L_j$ in the lower energy level is proportional to the electron number density of the corresponding energy level.

![Figure 1. Schematic of the principle of FIR temperature measurement based on thermal-coupled level.](image)

Therefore, the FIR of these two energy levels can be expressed by the following equation:

$$\text{FIR} = \frac{I_{2j}}{I_{1j}} = \frac{g_2 \sigma_{2j} \omega_{2j}}{g_1 \sigma_{1j} \omega_{1j}} \exp\left(-\frac{\Delta E_{21}}{k_B T}\right) = \frac{C \exp\left(-\frac{\Delta E_{21}}{k_B T}\right)}{g_2 \sigma_{2j} \omega_{2j}}$$

(2)

where $C=g_2 \sigma_{2j} \omega_{2j}/g_1 \sigma_{1j} \omega_{1j}$ [11] is a constant; $I_{2j}$ and $I_{1j}$, respectively, represent the fluorescence
intensities of the transition from $L_2$ and $L_1$ to $L_j$; $\sigma_{ij}$ and $\omega_{ij}$ represent the spontaneous emission probability and photon angular frequency of the corresponding radiation transitions, respectively.

As is evident from the above equations, the temperature of the material can be obtained through FIR. The formula is as follows:

$$\ln \text{FIR} = -\frac{\Delta E_{21}}{k_B T} + \ln C$$

From equation (3), the change rate of FIR can be used to characterize the temperature measurement performance. It is defined as absolute sensitivity $S_A$ and relative sensitivity $S_R$:

$$S_A = \left| \frac{d \left( \text{FIR} \right)}{dT} \right| = \frac{\Delta E}{k_B T}$$

$$S_R = \left| \frac{1}{\text{FIR}} \frac{d \left( \text{FIR} \right)}{dT} \right| = \frac{\Delta E}{k_B T^2}$$

Linearity, hysteresis, and repeatability are also important static characteristics of the sensor [11]. Linearity $\delta_L$ is as follows:

$$\delta_L = \pm \frac{\Delta Y_{\text{Max}}}{Y_{FS}} \times 100\%$$

where $\Delta Y_{\text{Max}}$ is the maximum deviation between the actual curve and the fitted line, and $Y_{FS}$ is the sensor full range output. Hysteresis $\delta_H$ is as follows:

$$\delta_H = \pm \frac{\Delta H_{\text{Max}}}{Y_{FS}} \times 100\%$$

where $\Delta H_{\text{Max}}$ is between the actual curves of forward.

3. Experimental Setup and Procedure

The temperature-dependent spectra of NaYF$_4$:Er$^{3+}$ UC under a 1550-nm excitation were studied using the FIR technique. The Fluorescence spectra at various temperatures were measured using an optical fiber probe. The probe was placed in a piece of isolated pig liver, which was injected NaYF$_4$:Er$^{3+}$ nanoparticle, the experimental setup was shown in figure 2.
The specific process of measuring the variable optical power and temperature spectrum is as follows: (1) Nano-particles NaYF₄:Er³⁺ was injected into isolated pig liver. (2) A 1550nm tunable laser was introduced into the isolated pig liver with a 600μm diameter optical fiber to heat the NaYF₄:Er³⁺ site. (3) A small spherical electronic thermometer temperature sensor was placed in this part, which was used to measure the temperature. (4) The reflection back upconversion fluorescence spectra of the optical fiber were recorded with a spectrometer. The variable temperature emission spectra of the NaYF₄:Er³⁺ UC phosphor within the temperature range of 298-318K were measured using a 1550nm tunable laser with a variable power of 5mW as the excitation source. In figure 2, the red arrow in the dashed frame represents the excitation light, which enters the fiber after passing through a filter with a high transmission 1550nm laser and high reflection visible light. The excitation light is transmitted to phosphor at the end of the optical fiber, and phosphor emits the UC fluorescence. The acquired fluorescence (green arrow) is transmitted back from the same optical fiber to the filter and reflected to the optical fiber spectrometer, which obtains the UC fluorescence spectra.

4. Results and Discussion

4.1. Fluorescence Spectra

Most of the UC luminescence of the rare-earth-doped NaYF₄ was used as the excitation light of 980nm [9, 12, 13]. However, recently excitation light at 1530nm has shown highly efficient UC emission [14]. In this study, a 1550nm tunable laser was used to investigate the optimal pumping wavelength of the sample by varying the pump wavelength and ensuring a constant pump light power. Figure 3 demonstrates the sample photoluminescence intensity versus the pump wavelength from 1515-1565nm at a pump power of 4mW. This shows that the optimal pumping wavelength is 1525nm.

As depicted in figure 3, the UC fluorescence spectra of the NaYF₄:Er³⁺ phosphor excited by a 1525nm laser were measured at 300K. These were composed of emission bands with peaks of 521nm (²H₁₁/₂→⁴I₁₅/₂), 541nm (²S₃/₂→⁴I₁₅/₂), and 655nm (⁴F₉/₂→⁴I₁₅/₂). The logarithmic relationship between the green and red luminescence intensities and the pump power (Iᵢ ∝ Pⁿ) are depicted in figure 4(a), where the slope of the fitting line represents the photon numbers to realize the UC process [10]. The slopes corresponding to the wavelengths of 521nm, 541nm, and 655nm are 2.127, 2.337, and 2.086, respectively, indicating that they are all three-photons absorption.
Figure 3. Fluorescence spectra of luminescence intensity of phosphor at room temperature based on pump wavelength.

Figure 4(b) depicts the energy level transition of the UC luminescence mechanism of Er^{3+} ions under 1525nm excitation. First, the Er^{3+} ions absorb a 1525nm photon from the ground state $^4I_{15/2}$ to the excited state $^4I_{13/2}$, and then continues to absorb another photon to the higher excited state $^4I_{9/2}$. However, Er^{3+} ions in the excited state of $^4I_{9/2}$, after non-radiation transition to the excited state of $^4I_{11/2}$, absorb the third photon to reach the state of $^4F_{9/2}$ before transitioning back to the ground state and radiate 655nm red light. They continue to absorb a 1525nm photon to reach the $^2H_{11/2}$ state, and transition to the ground state to produce green light at 521nm. The Er^{3+} ions in the excited state $^2H_{11/2}$ also transition to metastable state $^4S_{3/2}$ without radiation, and then transition to the ground state to produce green light at 541nm. The luminescence process is matched with the photon fitting process.

(a) Logarithmic curve of UC luminescence intensity and laser power of phosphor under excitation at 1525nm; (b) energy level transition of UC luminescence mechanism of Er^{3+} ions.

4.2. Up-Conversion Luminescence Spectra Dependence on Temperature

Using the experimental setup in figure 2, the up-conversion luminescence spectra of NaYF₄:2.5%Er³⁺ were obtained using a 1525nm laser pump, with the temperature ranging from 298K to 318K, at an optical power of 5mW. As depicted in figure 5, a normalized UC luminescence intensity of 521nm and 541nm emission bands is obtained on varying the temperature.
The FIR at various temperatures is depicted in Figure 6, which shows that the FIR increases with the temperature. After fitting, the value of $\Delta E/k_B$ was 1134, and the effective energy gap between $^2H_{11/2}$ and $^4S_{3/2}$ was approximately 787 cm$^{-1}$, which was close to the energy gap of absorption spectra of 710 cm$^{-1}$.

5. Conclusion
A tunable laser of C-band of optical communication was used to excite NaYF$_4$:Er$^{3+}$ phosphor in isolated pig liver. The UC luminescence intensity was the strongest at the wavelength of 1525nm. The luminescence spectra were measured in the temperature range of 298K-318K at 1525nm, which corresponding to the optical power of 5mW, through which the FIR of $^2H_{11/2}$ and $^4S_{3/2}$ energy levels ($I_{521}/I_{541}$) dependent on temperature were obtained. A 1550nm wavelength light source combined with
NaYF$_4$:Er$^{3+}$ with high efficiency up-convension fluorescent can be used to fabricate an up-convension FIR temperature sensor, which can have the potential applications in the experiment for further researching the killing of cancer cells by near-infrared laser.

**Acknowledgments**

This work was supported by Supported by Fujian Science Technology Innovation Laboratory for Optoelectronic Information of China (Grant No. 2021ZR141), the Science Foundation of Fuzhou City of Fujian Province of China (Grant No. 2020-GX-14), the Science Foundation of Fujian Province of China (Grant No. 2020Y4005).

**References**

[1] Sohail A, Ahmad Z, O Anwar Bégi, Arshad S, Sherin L. 2017 A review on hyperthermia via nanoparticle-mediated therapy[J] Bulletin du cancer 104 (5) 452-461.

[2] Letfullin R R, Letfullin A R and George T F. 2015 Absorption efficiency and heating kinetics of nanoparticles in the RF range for selective nanotherapy of cancer [J] Nanomedicine 11 412-420.

[3] Liu Z, Cai W B, He L N, Nakayama N, Chen K, Sun X M, Chen Y Y and Dai H J. 2007 In vivo biodistribution and highly efficient tumor targeting of carbon nanotubes in mice[J] Nat Nanotech 2 47-52.

[4] Chatterjee D K, Diagaradjane P and Krishnan S. 2011 Nanoparticle-mediated hyperthermia in cancer therapy[J] Ther Deliv 2 1001-1014.

[5] Gharouela S, Labrador-Páezb L, Haro-Gonzálezb P, Horchani-Naifer K and Férida M. 2018 Fluorescence intensity ratio and lifetime thermometry of praseodymium phosphates for temperature sensing[J] J Lumin 201 372-383.

[6] Wang X F, Liu Q, Bu Y Y, Liu C S, Liu T and Yan X H. 2015 Optical temperature sensing of rare-earth ion doped phosphors[J] RSC Advances 5 86219-86236.

[7] avchuk O A, Carvajal J J, Brites C D S, Carlos L D, Aguilo M and Diaz F. 2018 Upconversion thermometry: a new tool to measure the thermal resistance of nanoparticles[J] Nanoscale 10 6602-6610.

[8] Wang F, Banerjee D, Liu Y S, Chen X Y and Liu X G. 2010 Upconversion nanoparticles in biological labeling, imaging, and therapy[J] Analyst 135 1839-1854.

[9] Zhou S S, Deng K M, Wei X T, Jiang G C, Duan C K, Chen Y H and Yin M. 2013 Upconversion luminescence of NaYF$_4$:Yb$^{3+}$,Er$^{3+}$ for temperature sensing[J] Opt Commun. 291 138-142.

[10] Zhang H, Ye J J, Wang X L, Zhao S L, Lei R S, Huang L H and Xu S Q. 2019 Highly reliable all-fiber temperature sensor based on the fluorescence intensity ratio (FIR) technique in Er$^{3+}$/Yb$^{3+}$-co-doped NaYF$_4$ phosphors[J] J Mater Chem C 7 15269-15275.

[11] Collins S F, Baxter G W and Wade S A. 1998 Comparison of fluorescence-based temperature sensor schemes: theoretical analysis and experimental validation[J] J Appl. Phys 84 4649-4654.

[12] Feng Z H, Lin L, Wang Z Z and Zheng Z Q. 2019 NIR optical temperature sensing with efficiently relative sensitivity based on β-NaYF$_4$:Er$^{3+}$ nanoparticles[J] J LUMIN 221 117005.

[13] Feng Z H, Lin L, Wang Z Z and Zheng Z Q. 2021 Highly efficient and wide range low temperature sensing of upconversion luminescence of NaYF$_4$: Er$^{3+}$ nanoparticles: Effects of concentration of active or sensitive ions, excitation power and particle size on temperature sensing sensitivity[J] Opt Commun. 491 126942.

[14] Liu L, Lu K L, Xu L, Tang D Y, Liu C, Shahzad M K, Yan D, Khan F, Zhao E M and Li H Y. 2019 Highly efficient upconversion luminescence of Er heavily doped nanocrystals through 1530nm excitation [J] Opt Lett 44 711-714.