Introduction

Upconverting nanoparticles (UCNPs) have been developed as a new generation of imaging probes. Unlike commonly used quantum dots (QDs)\(^1\) UCNPs exhibit large anti-Stokes shifts in the near-infrared (NIR) spectral range where biological tissues are optically transparent.\(^2\) UCNPs also show sharp emission bandwidths,\(^3\) long emission lifetimes,\(^4\) tunable emission wavelengths,\(^5\) high photostability\(^6\) and relatively low cytotoxicity,\(^7\) which make them ideal candidates for cell labeling and imaging applications. Moreover, implementation of NIR light allows for an enhanced light penetration due to lower Rayleigh scattering as compared to the UV or the visible light excitation.

Upconverting Gd\(_2\)O\(_3\):Er\(^{3+}\),Yb\(^{3+}\),Zn\(^{2+}\) nanoparticles for biological applications: effect of Zn\(^{2+}\) doping\(^†\)

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Upconverting Gd\(_2\)O\(_3\) nanoparticles (NPs) doped 1% Er\(^{3+}\) and 18% Yb\(^{3+}\) permits one to perform optical imaging. Because of the presence of Gd\(^{3+}\) they are useful in MRI. The main challenge is to enhance the NPs upconversion efficiency. As a result of co-doping the NPs with Zn\(^{2+}\) ions, achieved using microwave-induced solution combustion synthesis, we obtained optimal upconversion quantum yields (UQYs). The breakdown of the local crystal field symmetry around the rare earth ions, maximal in the presence of 5% of zinc, may be responsible for the highest observed UQY. The upconversion of IR light results in emission of visible red light mainly at 660 nm and at 550 nm. Optimized red photoluminescence of the samples observed in an organic environment was examined as a function of the laser power density to explain the mechanism of the upconversion emission. Paramagnetic properties of the NPs were determined by superconducting quantum interference device measurements. The non-functionalized nanoparticles incubated with HeLa cells were endocytosed and imaged by confocal laser scanning microscopy. We investigated their localization inside HeLa cells for various incubation times and NPs concentrations. PrestoBlue toxicity assay was performed to test the NPs bio-efficacy.

S1, safety statement with the description of the microwave (Fig. S6 and S7), additional information of the parameters of the crystal lattice (Table S4, Fig. S8 and S9), TEM bright and dark field images of NPs (Fig. S10), size distribution of NPs (Fig. S11), quantum yields obtained at several different laser power (Tables S5 and S6), comparison of upconversion PL of Gd\(_2\)O\(_3\):Er\(^{3+}\),Yb\(^{3+}\),Zn\(^{2+}\) NPs obtained by the microwave-induced solution combustion synthesis with the homogenous precipitation method (Fig. S12), double logarithmic plots of the intensity dependencies of the up-converted fluorescence as a function of the excitation power green luminescence (non-calcined) and calcined samples (Tables S7 and S8, Fig. S13), luminescence decay curves of Er\(^{3+}\) (Fig. S14 and S15), summary of the luminescence decay of sample with different Zn\(^{2+}\) concentration (Tables S9–S14), S12: fluorescence maps of the NPs (Fig. S16), temperature dependence of reciprocal magnetic susceptibility for all the samples (Fig. S17), mass magnetization vs. T (Fig. S18), confocal images of HeLa cells after 4 h, 10 h, 24 h incubation in a solution: 1 µg mL\(^{-1}\) NPs, 25 µg mL\(^{-1}\) NPs, 40 µg mL\(^{-1}\) (Fig. S19–S21) are available. See DOI: 10.1039/c5ra11888c

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Several studies of NPs based on various oxide matrices (Y₂O₃, Lu₂O₃, La₂O₃, Gd₂O₃) doped with ions (Yb³⁺, Er³⁺, Tm³⁺ or Ho³⁺) were recently reported. These materials exhibit upconversion luminescence in the visible region (450–00 nm). Their useful optical properties arise from intra- (4f shell) and inter- (4f–5d shells) electronic transitions.

Contrary to most of the other rare earth ions, Gd³⁺ ions are paramagnetic and can effectively enhance magnetic resonance imaging (MRI). Unfortunately, up to now, MRI has not been utilized to its full potential for cancer diagnosis, mostly because of its low specificity (false-positive rate of 10% for breast cancer). The lack of MRI specificity can be, however, rectified using cell markers and unique properties, which can be utilized to enable detection with MRI in small quantities.

The main practical limitation with this type of material is its relatively low absolute quantum efficiency. The emission of the NPs depends on both: the phonon matrix energy and the activator crystal symmetry. An increase in luminescence intensity, due to enhanced emission quantum yield, is associated with lower crystal symmetry around the rare earth ions. This is achieved by modification of the surface in core/shell type NPs or by metal doping. Louis et al. synthesized doubly luminescent Gd₂O₃:Tb³⁺ NPs core encapsulated in a polysiloxane shell. The luminescence intensity in the NPs is greatly enhanced as compared to uncoated ones (core only). Kumar et al. reported that alkali-metal (Li⁺, Na⁺ or K⁺) doping of nano-sized Y₂O₃:Eu³⁺, can improve the luminescence intensity above the quenching concentration of Eu³⁺ ions. Double enhancement of the blue upconversion band for the Tm³⁺–Yb³⁺–Zn²⁺–Mg²⁺ co-doped with La₂O₃ phosphors has been observed by Kumari et al. Increasing the intensity of the green emission in the visible region by doping with Zn²⁺ ions was also found by Xu et al. in Gd₂O₃:Er³⁺ and Pandey et al. in Y₂O₃:Ho³⁺–Yb³⁺. The former author ascribed the reason for emission enhancement predominantly as a result of the lowered lattice crystal symmetry that resulted by introducing oxygen vacancy. The second author postulated that significant enhancement in the UC emission intensity was due to presence of the local field around the Ho³⁺ ions followed by the substitutional effect.

Quantum yield of UCNPs can be improved through three different approaches: content modification, introduction of the dopant ions into the crystal lattice at substitutional or interstitial positions, or changing the crystal field activator.

In this work, we study how the content of Zn²⁺ transition metal ions affected the absolute quantum yield of Gd₂O₃:1% Er³⁺,18% Yb³⁺ NPs. The Zn²⁺ concentrations reported in this study relate to the amount of Gd³⁺ in the starting materials. These materials were obtained using microwave-induced solution combustion synthesis, which generates the most efficient upconverting of NPs. In order to separate the NPs from each other during the microwave-induced solution combustion synthesis, NaCl was added. After the combustion process the NaCl was removed by dissolution in water. The resulted UCNPs were applied as luminescent markers in the biological materials. The NPs incubated with HeLa cells were found to be endocytosed by the cells. We examined their location inside HeLa cells for various incubation times and the NPs concentrations. To test material bioefficacy the toxicity test was performed by applying the PrestoBlue assay. The results indicate that the nanostructures are promising tags for bioimaging and biodetection.

In order to examine the morphology, distribution of elements, size, and crystal structure, the NPs were subjected to scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), and X-ray diffraction (XRD), respectively. The absolute quantum efficiency of NPs was measured using the integrating sphere technique. In addition, we measured cathodoluminescence (CL) spectra of the NPs, excited by an electron beam. The PL measurements were taken in the VIS and IR region using 980 nm laser excitation. With the use of the scanning confocal microscopy we determined decay times and obtained luminescence maps. Excitation–emission spectra of the NPs in powder form were measured using tunable Nd:YAG when excited from 900 nm to 1002 nm. Magnetic properties were measured using a superconducting quantum interference device at temperatures ranging from 5 K to 200 K. In order to determine the biological suitability of the NPs as a luminescent marker, a toxicity test was performed using PrestoBlue reagent. This reagent uses the reducing power of biological cells to quantitatively measure cell proliferation. In order to verify if these NPs can be applied as luminescent markers in vitro, the transfection agent Lipofectamine 2000 was used. The NPs were introduced into HeLa cancer cells and their luminescence was examined with a confocal laser scanning microscope. More details on the measurement methods is provided in the ESI (SI1).

Results and discussion

Characterization

In this study, we successfully synthesized Gd₂O₃ NPs doped with 1% Er³⁺ and 18% Yb³⁺ and various concentrations of Zn²⁺ (0%, 2.5%, 5%, 20%, 25%) in the input materials using microwave-induced solution combustion synthesis (Table S1, SI1). Three independent samples of Gd₂O₃:1% Er³⁺,18% Yb³⁺ nanoparticles were prepared to test the structural reproducibility of the XRD determinations (Fig. S4, Table S2 (SI1)). In addition, emission spectra of Gd₂O₃:1% Er³⁺,18% Yb³⁺,5% Zn²⁺ nanoparticles were determined on three independent samples (Fig. S5, Table S3 (SI1)).

All experiments involving microwave-induced solution combustion synthesis were performed maintaining required safety standards (Fig. S6 and S7 (SI1)).

The results of laboratory X-ray diffraction measurements performed at RT on doped Gd₂O₃ NPs samples have shown that all studied materials are mixtures of two gadolinium(III) oxide structure types: the monoclinic one (space group C2/m) and the cubic one (space group Ia3). The un-doped sample and samples with low Zn content (x = 2.5% and x = 5%) do not show presence of any foreign phase. The middle zinc doped samples (x = 20% and x = 25%) additionally include a trace amount of crystalline hexagonal ZnO. The highly doped sample (x = 50%) contains zinc oxide at the level of 9.5(2) wt%. Using the Rietveld refinement procedure we determined the lattice parameters of...
all studied samples and their quantitative composition (Fig. S8 and S9, (SI1)†). The lattice parameters for the investigated samples are shown in Table S4 (SI1).† An example of Rietveld refinement results for an un-doped sample and for a Zn doped one (x = 5%) are shown in Fig. 1a and b. The runs of relative values of lattice parameters (l/\(l_0\)) against the nominal x value (see Fig. 1c) clearly demonstrate that Zn doping causes a decrease of values of all lattice parameters, for both Gd₂O₃-based phases, and that the solubility limit of Zn in Gd₂O₃ is around 5%. We do not exclude that some part of the zinc oxide entering into reaction is present in the form of nanocrystallites which are hardly visible. A slight increase of relative lattice parameters values for the Zn enhanced concentration (from 25% to 50%) is probably due to the homogeneity in a crystal lattice generated by interstitial Zn²⁺ ions and high concentrations of oxygen vacancies.

Anyway, for subjects of the present work, we observed that replacement of Gd³⁺ by Zn²⁺ causes a contraction of the crystal lattice of the Gd₂O₃:1% Er³⁺,18% Yb³⁺ NPs doped up to 5% content of Zn²⁺. Size of the unit cell decreases linearly and lengths of bonds between the Gd³⁺ ions and erbium, ytterbium also ions decreased which causes the breakdown of the local crystal field symmetry around the RE ions. A related behavior was reported by Lin et al.28 for NaYF₄:Yb³⁺/RE³⁺ (RE = Tm, Ho) microcrystals via Li⁺ doping.

The EDX method was applied to check the absence of foreign elements in the tested samples (Fig. 2). As judged by the results, we obtained approximately similar atomic ratios of Gd³⁺/Yb³⁺, Gd³⁺/O in the samples containing three different zinc concentrations (data not shown). Determination of atomic ratios of Gd³⁺/Er³⁺, Yb³⁺/Er³⁺ are not accurate, due to low concentrations of zinc and erbium (below the instrumental detection limit of 1%). This precludes a precise quantitative analysis.

Transmission electron microscopy (TEM) bright and dark field images of NPs are shown in Fig. S10a–j (SI1).† The NPs crystallites with an estimated size of 33–52 nm are observed. Histograms of the size distributions for the NPs are shown in Fig. S11a–e (SI1).†

**Optical properties**

We applied the microwave-induced solution combustion synthesis because it generates the oxide materials with the highest upconversion efficiency. Fig. S12† shows comparison of the spectrum Gd₂O₃:Er³⁺,Yb³⁺ UCNPs obtained by microwave-induced solution combustion (Fig. S12a, SI1†) and the precipitation method29 (Fig. S12b, SI1†). The latter NPs was calcined 3
in air at a temperature of 900 °C. The high temperature (>1500 °C [ref. 30]) microwave-induced solution combustion method allows one to obtain a two orders of magnitude higher upconversion intensity compared with the precipitation method. The method is repeatable and suitable for a useful synthesis (a few grams per process) of the NPs. The predominant disadvantage of the first method is partial agglomeration of the NPs. To prevent this, we applied NaCl in the process of synthesis.26 In the second method we applied a lower temperature for obtaining nanoparticles. This resulted in smaller crystallites and consequently a lower upconversion intensity.

**Absolute quantum yield of Gd₂O₃:Er³⁺,Yb³⁺ luminescence for various Zn²⁺ concentrations**

Absolute quantum yield measurements of NPs were performed using the integrating sphere technique. The following formula describes the absolute quantum yield:

\[
QY = \frac{I_{emission}}{I_{reference} - I_{excitation}}
\]

where: \(I_{emission}\) is an area under the emission curve of the sample, \(I_{reference}\) is an area under the emission curve scanned over the excitation peak in the absence of the fluorescence sample, and \(I_{excitation}\) is an area of the excitation peak in the presence of the sample.

The highest quantum efficiency was obtained for the 5% concentration of zinc. The PL quantum yield was designated as a function of excitation power. The highest quantum yield was 0.09% at 980 nm excitation wavelength. The continuous wave (cw) laser power density was 19 W cm⁻² (Fig. 3a, Table S5 [SI1]). We also measured the absolute quantum efficiency (eqn (1)) of the Gd₂O₃:1% Er³⁺,18% Yb³⁺ NPs with 5% of Zn²⁺ for various concentrations of NaCl: 3 mM, 6 mM, 8 mM and 11 mM. NPs were trapped in the NaCl matrix which prevented the agglomeration of the NPs. A similar effect was observed by others during the synthesis of mesoporous ceria–zirconia31 or CoFe₂O₄ (ref. 32) and Fe₂O₃ (ref. 33) NPs.

In the present work, in the case of the NPs synthesized with added NaCl, quantum efficiency was 0.0044% for 3 mM NaCl in the sample. With increasing amounts of the NaCl in the sample, the quantum efficiency decreased at power density of 19 W cm⁻² (Fig. 3b, Table S6 [SI1]), whereas quantum efficiency was the highest for the nanoparticles without salt.

**Fig. 2** Energy dispersive X-ray spectrum of the NPs samples with various concentrations of Zn²⁺: (a) 0%, (b) 5% and (c) 50%.

**Fig. 3** Dependence of the quantum efficiency as a function of excitation power density: (a) Gd₂O₃:1% Er³⁺,18% Yb³⁺ NPs for different zinc content (non-calcined samples) (b) the NPs for the 5% Zn²⁺ for different molar salt concentrations. In both cases the samples were non-calcined. The spot diameter was approximately 2 mm.
The CL spectra of the NPs excited by an electron beam (accelerating voltage of 15 kV; filament probe of 1 nA) are shown in Fig. 4a and b. As previously reported (Gao et al.34), the relative intensity of the peaks obtained by PL and CL differed most likely due to different excitation mechanisms. A series of specific transitions for Er3+: 4H9/2 → 4I15/2, 4F7/2 → 4I15/2, 2S3/2 → 4I15/2 and 4F9/2 → 4I15/2, corresponding to wavelengths 417 nm, 488 nm, 535 nm and 660 nm (Fig. 4a), were observed. In the region from 750 nm to 1050 nm the peaks at the wavelengths: 954 nm, 977 nm and 1027 nm corresponding to Yb3+ transitions 2F5/2 → 2F7/2 (1–6), 2F5/2 → 2F7/2 (5–1) and 2F5/2 → 2F7/2 (5–3) (Fig. 4b) were noted. The highest intensity of the CL in this spectral region from 900 nm to 1050 nm was noted for the NPs sample with 5% Zn2+ concentration.

Enhancing of Gd2O3:Er3+,Yb3+ luminescence by Zn2+

Intensity dependencies of the fluorescence, as a function of the 980 nm excitation power obtained for non-calcined and calcined samples, are shown in Fig. 5 and S9 (SI1).† PL spectra were integrated, to clarify the UC mechanism and the dependence of the visible emission on the pump power; the pump-power-dependent UC behavior of all emission bands was investigated. The UC luminescence intensity (Iup) is related to the pump power at 980 nm (P) through the following formula:35

\[ I_{\text{up}} \sim P^n \]

where \( I_{\text{up}} \) is the upconversion luminescence intensity and \( P \) is the pump power. The exponent \( n \) is integer number of photons absorbed in the upconversion emission process. Values of \( n \) for each sample are denoted in Fig. 5 and S9 (SI1).† The emission bands can be assigned to intra-4f electronic transitions of the Er3+ ions. Fig. 5a and b and Tables S7 and S8 (SI1)† shows the \( n \) values for transitions 4F9/2 → 4I15/2 (red emission) in the range from 630 nm to 700 nm for non-calcined and calcined samples. Fig. S1 and b (SI1)† shows the \( n \) values corresponding to transitions 4H9/2 → 4I15/2 and 4S1/2 → 4I15/2, corresponding to wavelengths 510 nm to 585 nm for non-calcined and calcined samples.36 (Tables S7 and S8 (SI1)†). These results show that the emissions are two-photon processes for a Yb/Er pair. The upconversion mechanisms between the Yb3+/Er3+ pairs were described previously by Kamińska et al.37 and Sikora et al.38

Some saturation of the upconversion process was observed previously for the powdered Y2O3 materials doped with Er3+/Yb3+ (Singh et al.40). In such cases, at very high pump power, the up-converted intensity became independent of the pump intensity. The emission intensity of the sample with 25% Zn2+ concentration in the range 510–585 nm, at the lower power range is linear with the slope \( n = 0.56 \), and for higher laser power the slope changes to 2.47 (Fig. S13a, Table S7 (SI1)).† It was also observed by Singh et al.40 for Er3+/Yb3+ co-doped Y2Ti2O7. Materials investigated in this work exhibited emission at 548 nm and 661 nm. The power...
dependence of the green bands upconversion intensity at low laser power were described with \( n = 0.59 \), and for slightly higher laser power, \( n = 2.2 \). Other slopes were observed in the case of non-calcined and calcined for 2 h samples with 50% Zn\(^{2+}\). The slope of the region at wavelengths from 510 nm to 585 nm for low laser power were \( n = 1.82 \) and \( n = 1.96 \), for 50% Zn\(^{2+}\) and 50% Zn\(^{2+}\) (calcined) samples, respectively. The slopes gradually decreased to 1.54 (50% Zn\(^{2+}\)) and 0.92 (50% Zn\(^{2+}\) (calcined)) samples which could be attributed to the local thermal effects induced by the laser exposure;\(^{44,42}\) see (Fig. S13a and b (SI1)?) (Tables S7 and S8 (SI1)). After increasing the laser power, thermal equilibrium occurred between different energy levels, which was related to lowering emissions and a decrease in the slopes.\(^{44}\) A similar behavior was observed in YAO\(_3\) crystals doped with Ho\(^{3+}\) and Yb\(^{3+}\).\(^{43}\)

**Effect of Zn\(^{2+}\) concentration in Gd\(_2\)O\(_3\):(1% Er\(^{3+}\),18% Yb\(^{3+}\)) on the upconversion efficiency**

The PL spectra of Gd\(_2\)O\(_3\):1% Er\(^{3+}\),18% Yb\(^{3+}\) powdered NPs doped with different concentrations of Zn\(^{2+}\), at two different laser power densities (1.5 W cm\(^{-2}\) and 31.0 W cm\(^{-2}\)), are shown in Fig. 6a and b, respectively. An increase of the green luminescence for a power density of 31.0 W cm\(^{-2}\) was noted. This increase can be attributed to the thermal effect, which is caused by the exposure of the 980 nm diode laser continuous wave (cw) laser power density. Similar observations were reported by Lei et al.\(^{44}\) for Gd\(_2\)O\(_3\):Er\(^{3+}\),Yb\(^{3+}\) nanowires. For large Yb\(^{3+}\) concentrations in the oxide matrix and a higher NIR laser power excitation, more NIR photons were absorbed, leading the temperature increase.

The highest intensity of the luminescence was observed for a sample with 5% Zn\(^{2+}\) for both laser power densities. The spectra are composed of two clearly distinct bands: the first at a wavelength ~550 nm and a second at a wavelength of ~660 nm, characteristic for the upconversion process, as reported previously by Kamińska et al.\(^{27}\) This observation is consistent with our absolute quantum yield estimations (Fig. 6). A relative number of photons for the characteristic transitions \(^{4}F_{9/2} \rightarrow ^{4}I_{15/2}\) (green emission) and \(^{4}F_{9/2} \rightarrow ^{4}I_{15/2}\) (red emission), depended on zinc concentration at 1.5 W cm\(^{-2}\) (Fig. 6c and e) and 31.0 W cm\(^{-2}\) (Fig. 6d and f) power density. A relative number of photons involved in the green emission, for the sample with 5% Zn\(^{2+}\) at 31.0 W cm\(^{-2}\) the laser power density, was 80 times higher than at the power density of 1.5 W cm\(^{-2}\). At the same time, with a relative number of photons of the red emission for the sample at 31.0 W cm\(^{-2}\), the laser power density is 5 times higher than at a power density of 1.5 W cm\(^{-2}\). We also measured PL of the NPs in the infrared region at listed laser power densities (Fig. 6g and h). We observed specific transitions between energy levels for the Yb\(^{3+}\) i.e. from the \(^{2}F_{7/2}\) to \(^{2}F_{5/2}\) level, corresponding to a wavelength of 1030 nm. In Gd\(_2\)O\(_3\) NPs doped Er\(^{3+}\) and Zn\(^{2+}\) synthesized via a citric acid complexing method, Xu et al.\(^{44}\) showed an increase in green luminescence with Zn\(^{2+}\). In our case, the NPs in this work, exhibits an increase in red luminescence at a 5% Zn\(^{2+}\) concentration at both power densities. The oxide matrix has a higher phonon energy than NaYF\(_4\) (597 cm\(^{-1}\) vs. 360 cm\(^{-1}\)). A large lattice phonon energy is usually helpful to bridge the larger gap for the red emission.\(^{45,46}\) The Yb\(^{3+}\) excitation in the Gd\(_2\)O\(_3\):(Er\(^{3+}\),Yb\(^{3+}\),Zn\(^{2+}\)) material seems to be more effective than pumping Er\(^{3+}\) in view of the relatively small absorption cross section of Er\(^{3+}\) ions at 980 nm.\(^{47}\) It is also observed that, in the case of the sample without Zn\(^{2+}\) ions, the relative number of photons, or transitions between levels of Er\(^{3+}\) responsible for the red luminescence, is 7.5 times lower compared with the sample containing 5% Zn\(^{2+}\) at a laser power density of 1.5 W cm\(^{-2}\) and 13 times lower than the sample at a power density of 31.0 W cm\(^{-2}\). For the sample without Zn\(^{2+}\) ions no observable photons or transitions between levels of Er\(^{3+}\) responsible for the green luminescence at a laser power density of 1.5 W cm\(^{-2}\) were noted. However, at 31.0 W cm\(^{-2}\) power, 13 times lower compared with the sample (5% Zn\(^{2+}\)), the relative number of photons or transitions between levels of Er\(^{3+}\) responsible for the green luminescence was observed.

There are several theories which explain the enhanced emission of upconverting materials after doping with Zn\(^{2+}\). The first explanation can be lowering the crystal symmetry of gadolinium oxide by introducing oxygen vacancies. Incorporation of Zn\(^{2+}\) as dopant (electronegativity, \( \chi \) : 1.65) reduces interactions between Yb\(^{3+}/Er^{3+}/Gd^{3+}\) and with oxygen O\(_2\). The electronegativity values for Er\(^{3+}\), Yb\(^{3+}\), Gd\(^{3+}\) ions are 1.24, 1.1, 1.2, respectively.\(^{24,48}\) The function of charge and compensation of the oxygen vacancies can be distinguished, due to the difference in electronegativity, between zinc ions Zn\(^{2+}\) and the rare earth ions in the Gd\(_2\)O\(_3\) host matrix. In our study, the Kröger–Vink notation was applied for the determination of oxygen vacancies arising in doped phosphors:\(^{44}\)

\[
\text{Gd}_2\text{O}_3 \xrightarrow{\text{Zn}^{2+}} 2\text{Zn}_{\text{od}} + \text{V}_o + 3\text{O}_5^-
\]

where Zn\(_{\text{od}}\) represents ion state Zn\(^{2+}\), which substitutes Gd\(^{3+}\) ion in the crystal lattice, \( \text{V}_o \) represents the oxygen vacancies, and \( \text{O}_5^- \) represents oxygen in the crystal lattice.

On the other hand, the sensitization effect, either generated directly by the presence of Zn\(^{2+}\) or due to the local field around the erbium ions caused by the Zn\(^{2+}\), could be responsible for the enhanced UC. In the case of Zn\(^{2+}\) doping Y\(_2\)O\(_3\):(Ho\(^{3+}\),Yb\(^{3+}\)) phosphor, Pandey et al.\(^{45}\) observed significant enhancement of UC emission. They observed a change in decay time attributed to the local field effect which arises due to co-doping of Zn\(^{2+}\) ions.\(^{40}\) In our case, we did not observe such effect. However, Fig. 7 shows the luminescence decay times for the samples with transitions in Er\(^{3+}\): \(^{4}S_{3/2} \rightarrow ^{4}I_{15/2}\) (green emission) and \(^{4}F_{9/2} \rightarrow ^{4}I_{15/2}\) (red emission). Decay curves fit to a single exponential function: \( y = A \exp(-t/\tau) \), where \( A \) is the initial emission intensity at \( t = 0 \), and \( \tau \) is the lifetime (Fig. S14 and S15 (SI1)). Average luminescence decay time of the samples were summarized in Table 1. The value of the average decay time for Er\(^{3+}\) red emission is maintained at the same level with Zn\(^{2+}\) concentration increase, with the exception of the sample with 50% Zn\(^{2+}\). In addition, the value of the average decay time for the red transitions Er\(^{3+}\) emission is higher, compared with the green emission of Er\(^{3+}\) for all tested samples, as described in
Fig. 6  PL spectra of NPs doped with different amounts of Zn$^{2+}$ at (a) 1.5 W cm$^{-2}$ and (b) 31 W cm$^{-2}$ laser power density. Relative number of photons for the specific transitions $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ (green emission) and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ (red emission) depending on the concentration of zinc at (c) 1.5 W cm$^{-2}$ and (d) 31 W cm$^{-2}$ laser power density. Relative number of photons for the transitions $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ (green emission) and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ (red emission) region depending on particle size determined by TEM and the laser power density at (e) 1.5 W cm$^{-2}$ and (f) 31 W cm$^{-2}$. PL spectra of all samples in the infrared region at (g) 1.5 W cm$^{-2}$ and (h) 31 W cm$^{-2}$ laser power density.

Fig. 7, Table 1. The same dependence was reported by Zheng et al. in the case of spherical Gd$_2$O$_3$:Yb$^{3+}$/Er$^{3+}$@SiO$_2$ particles obtained by heterogeneous precipitation method. Individual measurements of the luminescence decay were shown in Tables S9–S14 (SI1).† We determined fluorescence maps of Gd$_2$O$_3$:1% Er$^{3+}$,18% Yb$^{3+}$ NPs with various Zn$^{2+}$ concentrations at 550 nm
understand the UC process we examined intensity dependences of the upconverted fluorescence as a function of the excitation power at 980 nm (see Fig. 7).

Liu et al., postulated that enhanced luminescence efficiency could be a result of the Zn$^{2+}$ flux effect. The authors believe that it improves crystallization, enhanced grain size, and also reduces the luminescence quenching due to the surface states. Kumari et al., in La$_2$O$_3$ phosphors doped/co-doped with Tm$^{3+}$ and Yb$^{3+}$ and Zn$^{2+}$/Mg$^{2+}$ synthesized by a urea assisted solution combustion technique, reported enhancement of the UC emission intensity and attributed it to the effect of the broadening of the absorption bands, followed by the overlap between the defect states produced by the co-dopants with the RE ions charge transfer states.

Additionally, the enhanced emission of upconverting materials was observed by others with fluoride matrices. Tu et al., presented upconversion luminescence enhancement strategy, in aqueous media, based on the surface plasmon resonance (SPR) between NaYF$_4$:Yb$^{3+}$/Er$^{3+}$ and gold nanoparticles. Deng et al., reported a facile in situ cation exchange strategy to greatly enhance the UC luminescence of hexagonal phase NaYF$_4$ NPs while maintaining their small particle size and shape. Via a cation exchange treatment by hot-injecting Gd$^{3+}$ precursors into the NPs solution without pre-separation, the naked-eye visible UC emission of the NPs was enhanced about 29 times under 980 nm near infrared (NIR) excitation with unchanged particle size.

In this work, in the case of Gd$_2$O$_3$:1% Er$^{3+}$,18% Yb$^{3+}$ NPs with various Zn$^{2+}$ content, upconversion luminescence is found to be optimal in the presence of 5% content of Zn$^{2+}$. The results support the theory proposed by Lin et al., who postulated that the transitions will occur due to the asymmetric environment surrounding RE ions, subsequently resulting in the enhanced UC luminescence. Normalized excitation–emission spectra for the NPs with 2.5% and 50% Zn$^{2+}$ concentrations are shown in Fig. 8a and b. The differences in the peak intensity ratios are observed for the NPs with 2.5% Zn$^{2+}$. The excitation–emission spectra of this sample exhibit higher intensity in comparison with the 50% Zn$^{2+}$ NPs in the range from 920 nm to 980 nm. In both samples the effect is dominated by fluorescence with an excitation maximum of 980 nm, which is also present in the PL excitation maps (Fig. 8c and d).

The optimized red photoluminescence spectra of Gd$_2$O$_3$:1% Er$^{3+}$,18% Yb$^{3+}$,5% Zn$^{2+}$ NPs in an organic environment are presented in Fig. 9a. The NPs were dissolved in dimethyl sulfoxide (DMSO) and the PL spectra were measured at excitation of 980 nm (Fig. 9b). The sample with 5% Zn$^{2+}$ concentration emits green light from 519 nm to 574 nm and red light from 636 nm to

and 660 nm (Fig. S16 (SI2)†). All images were acquired for the same sample area excited at 980 nm. The stronger luminescence in the red channel for all samples was observed. In the case of red luminescence, excluding the sample with 50% Zn$^{2+}$ concentration, gradual increase of Zn$^{2+}$ concentration caused excessive generation of oxygen vacancies which affected the non-radiative decay rates. This leads to suppression of the luminescence and shortening of its lifetime.

Fig. 7 The luminescence decay time for Gd$_2$O$_3$:1% Er$^{3+}$,18% Yb$^{3+}$ NPs with different amount of Zn$^{2+}$ for the transitions $^4S_{3/2} \rightarrow ^4I_{13/2}$ (green emission) and $^4F_{9/2} \rightarrow ^4I_{13/2}$ (red emission) at excitation wavelength of 980 nm.

Fig. 8 Normalized photoluminescence emission intensity at various excitation energies of Gd$_2$O$_3$ NPs doped 1% Er$^{3+}$,18% Yb$^{3+}$ (a) 2.5% Zn$^{2+}$ and (b) 50% Zn$^{2+}$. Maps joining excitation and emission spectroscopy Er$^{3+}$–Yb$^{3+}$ centers in the (c) 2.5% Zn$^{2+}$ and (d) 50% Zn$^{2+}$ samples.

Table 1 Average luminescence decay time for transitions $^4S_{3/2} \rightarrow ^4I_{13/2}$ (green emission) and $^4F_{9/2} \rightarrow ^4I_{13/2}$ (red emission)

<table>
<thead>
<tr>
<th>The content of Zn$^{2+}$ in relation to the amount of Gd$^{3+}$ in the starting material Gd$_2$O$_3$:1% Er$^{3+}$,18% Yb$^{3+}$ (%)</th>
<th>Average green luminescence lifetime (µs) ($^4S_{3/2} \rightarrow ^4I_{13/2}$, 550 nm)</th>
<th>Average red luminescence lifetime (µs) ($^4F_{9/2} \rightarrow ^4I_{13/2}$, 660 nm)</th>
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Magnetic properties

Independently of the zinc content, the NPs exhibit paramagnetic behavior. Representative zero-field-cooled (ZFC) and field-cooled (FC) temperature dependences of magnetization are shown in Fig. 10a. For each sample both curves are almost superimposed, as shown in Fig. 10a, and obey the Curie–Weiss law \( \chi = C/(T - \Theta) \), where \( \chi \) is magnetic susceptibility, \( C \) is Curie constant, \( T \) is absolute temperature, and \( \Theta \) is paramagnetic Curie temperature. Analysis of the reciprocal magnetic susceptibility clearly revealed antiferromagnetic contribution (Fig. S17 (SI2)†). The paramagnetic behavior for the whole set of the samples was also confirmed by the field dependent magnetization. Fig. 10b shows straight, linear \( M(H) \) function at fields up to 400 Oe, indicating behavior typical for paramagnets. A similar behavior was observed for undoped \( \text{Gd}_2\text{O}_3 \) NPs.\(^\text{38}\) Mass magnetization vs. \( T \) for seven samples is shown in Fig. S18a (SI2).† Estimated values of \( \Theta \) show an upward trend with increasing \( \text{Zn} \) content (Fig. S18b (SI2)†). In the case of FC data, changes from \(-13.6 \, \text{K} \) to \(-9.8 \, \text{K} \), for the samples with \( \text{Zn}^{2+} \) content from 0% to 50% are observed. The paramagnetic Curie temperature, taken from ZFC dependencies, slightly differ (lowered by up to 1 K) from these obtained from FC data. However, the latter qualitatively reproduces the former. These values are lower than for the \( \text{Gd}_2\text{O}_3 \) bulk material in both expected crystal structures, \( i.e. \) around \(-17 \, \text{K} \) for the cubic phase and even \(-30 \, \text{K} \) for the monoclinic phase.\(^\text{59} \) However, recent studies of cubic phase \( \text{Gd}_2\text{O}_3 \) powder show values of \( \Theta \) from \(-3 \, \text{K} \) to 0 K.\(^\text{47,60,61} \)

Toxicity test of NPs

To test the material biocompatibility, PrestoBlue cytotoxicity assays were performed. The results in Fig. 11 confirmed that gadolinium oxide NPs, with erbium, ytterbium and zinc polyvinyllpyrrolidone (PVP) coated are highly biocompatible at the NPs concentration of up to 50 mg mL\(^{-1} \) after 24 h incubation. PVP was selected as a capping agent\(^\text{46} \) to inhibit nanoparticle aggregation and to make them hydrophilic and non-toxic. After incubation with the NPs for 48 h the cell viabilities were still greater at 90%, and decreased slightly to 68% (50 mg mL\(^{-1} \)). These results imply that the NPs demonstrates good

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**Fig. 9** (a) The spectra of the 0.02 mg mL\(^{-1} \) NPs in DMSO solution as a function of laser power density. (b) Visual demonstration of the optimized red luminescence of the NPs in an organic environment. (c) Relative number of photons for the transitions \( ^{5}S_{3/2} \rightarrow ^{1}I_{15/2} \) (green emission) and \( ^{4}F_{9/2} \rightarrow ^{4}I_{15/2} \) (red emission) as a function of laser power density in DMSO solution. (d) Intensity ratio of green emission to the red emission as a function of laser power density for the sample with 5% \( \text{Zn}^{2+} \) concentration in the DMSO environment.

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**Fig. 10** (a) ZFC and FC magnetization and corresponding reciprocal susceptibilities as a function of temperature for the sample with the highest \( \text{Zn}^{2+} \) content. (b) Magnetic field dependence of magnetization for all samples.

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**Fig. 11** Viability of HeLa carcinoma cells after 24 h and 48 h incubation with five concentrations of 5% \( \text{Zn}^{2+} \) NPs sample as determined by the Presto Blue assay.
biocompatibility, which is highly desirable for future cell imaging applications. Other investigators also checked the toxicity of materials based on the Gd$_2$O$_3$ matrix. Zhou et al. received a good biocompatibility silica coated Gd$_2$O$_3$:Yb$^{3+}$,Er$^{3+}$/Tm$^{3+}$ NPs of HeLa cells incubated for 2 h. Hemmer et al. examined the cytotoxic effect of bare and poly(ethylene glycol)-b-poly(acrylic acid) block co-polymer-modified up-converting Gd$_2$O$_3$:Er$^{3+}$,Yb$^{3+}$ nanostructures (NPs and nanorods) on non-phagocytic and phagocytic cells (B-cell hybridoma cells and macrophages). The observed cytotoxic behavior in the case of macrophages incubated with bare nanostructures was assigned to the poor chemical durability of gadolinium oxide, but could be overcome by surface modification. Liu et al. demonstrated non-cytotoxic character in PEGylated Gd$_2$O$_3$:Yb$^{3+}$,Er$^{3+}$ nanorods.

Cell culture and laser scanning luminescence microscopy imaging in vitro

Several groups investigated cell cultures incubated with different concentrations of nanomaterials based in Gd$_2$O$_3$. Liu et al. synthesized PEGylated Eu$^{3+}$ doped mesoporous
gadolinium oxide nanorods that were incubated 24 h in two cell cultures: HepG2 (derived from liver cancer cells) and MCF-7 (human breast cancer line). As a result of such incubation, no changes were observed in the morphology of cell nuclei. High biocompatibility and showing feasibility for disease diagnosis and chemotherapy was proposed by the authors. On the other hand, in 2013 Liu et al. received PEGylated Gd2O3:Yb3+,Er3+ nanorods and incubated the material with the above cell lines for 48 h. It was found that all cells spread and proliferated equally, reflecting no differences between the two groups in cellular morphology. Lechevallier et al. tested the internalization of Gd(OH)2CO3:Eu3+ and Gd2O3:Eu3+ NPs prepared via urea-assisted precipitation, using HeLa cells (12 h incubation). Both types of NPs were internalized. A red emission has been observed in the cytoplasm, being more intense when closer to the nucleus.

In the present paper, the Gd2O3:1% Er3+,18% Yb3+,50% Zn2+ concentration, PVP coated NPs were also introduced into HeLa cancer cells using the procedure described previously by Kamińska et al. Solutions were prepared with various concentrations of NPs: 1 μg mL⁻¹, 25 μg mL⁻¹, 40 μg mL⁻¹ and 50 μg mL⁻¹, then incubated with HeLa cells for different times: 4 h, 10 h, and 24 h. Next, the cells were fixed for microscopic preparations. The presence of the NPs in the cells was confirmed using a confocal microscope. After excitation by a femtosecond pulse of NIR radiation, we observed strong UC luminescence in cells without apparent autofluorescence. Confocal images of HeLa cells after 4 h, 10 h, and 24 h incubation in a solution 50 μg mL⁻¹ Gd2O3:1% Er3+,18% Yb3+,50% Zn2+/PVP NPs with Lipofectamine 2000 are shown in Fig. 12a–c. The remaining confocal images of HeLa cells with 1 μg mL⁻¹, 25 μg mL⁻¹, and 40 μg mL⁻¹ are presented in Fig. S19 and S21 (SI2).† The increase of concentration and incubation time enhanced the number of NPs in the cytoplasm or on the cell membrane (Fig. 12a–c and S19–S21 (SI2)).† The spectra of the sample with 50% Zn2+ concentration, PVP coated NPs, collected after: 4 h incubation in HeLa cells, 40 μg mL⁻¹ (concentration NPs) and 0.6% maximum laser power (0.006 W), are shown in Fig. 13a; 24 h incubation in HeLa cells and 25 μg mL⁻¹, 1% maximum laser power (0.01 W) are shown in Fig. 13b. In each case, a signal from the NPs was collected in the range from 489 nm to 718 nm. We also observed two peaks, similar to PL measurements (cw laser), derived from 4F9/2 → 4I15/2 transition with Er3⁺ ions in maximum at a wavelength about 550 nm and 660 nm.

Conclusions

Gd2O3(Er3⁺,Yb3⁺) NPs doped with various concentrations of Zn2⁺ were successfully synthesized by a microwave-induced solution combustion synthesis. X-ray diffraction studies revealed, in the case of non-calcined samples, that the NPs are dominated by a monoclinic phase with contributions of a cubic phase. For calcined samples the quantitative content of the cubic phase was greater than the monoclinic one. A reduced symmetry center of Er3⁺ was associated with a higher photoluminescence intensity. The highest quantum yield was 0.09% upon 980 nm excitation (cw, 19 W cm⁻²) for a sample containing 5% Zn2⁺ in relation to the amount of Gd³⁺ in the starting material. The NPs diameter was 46.0 ± 0.5 nm, as determined by TEM. Logarithmic dependence of the emission intensity for gadolinium oxide (III) doped Er³⁺,Yb³⁺ with Zn²⁺ input NPs as a function of laser power in the regions of the red luminescence (630–700 nm) and of the green luminescence (510–585 nm) indicates a two-photon absorption process. Average decay time for the Er³⁺ red emission was maintained at the same level with the increase in the Zn²⁺ concentration (44 µs to 47 µs) and was 76 µs for the NPs with 50% Zn²⁺. Optimized NPs, dissolved in DMSO, exhibit significant increase in the red luminescence with increasing laser power density at 980 nm wavelength. Paramagnetic behaviour of NPs was confirmed by a temperature dependence of magnetization. Our results indicate that the NPs are not toxic at the selected concentrations up to 50 μg mL⁻¹. It has been shown that the NPs, in the presence of liposomes, accumulate in the cytoplasm. The nanoparticles can be used as promising labels for bioimaging and biodetection.

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Notes and references


