Hybrid upconverting/magnetic $Fe_3O_4/Gd_2O_3:Er^{3+},Yb^{3+}, Mg^{2+}, Nd^{3+}$ nanoparticles – synthesis, characterization and biological applications

I. Kamińska[1]*, K. Sobczak[2], Y. Zhydachevskyy[1], T. Wojciechowski[1,3], R. Minikayev[1], B. Sikora[1], S. Lewińska[1], M. Chojnacki[1,3], K. Fronc[1,3]

[1] Institute of Physics Polish Academy of Sciences, al. Lotników 32/46, 02-668 Warsaw, Poland,
[2] Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland,
[3] International Research Centre MagTop, al. Lotników 32/46, 02-668 Warsaw, Poland

emial: ikaminska@ifpan.edu.pl*

INTRODUCTION

Researchers are very interested in nanoparticles (NP) doped with lanthanides that show the anti-Stokes emission called upconversion (UC). These NPs can be excited by near infrared (NIR) 980 nm light, which penetrates into biological tissues to a depth of several cm. In addition, thanks to the use of such excitation medium, we do not observe autofluorescence of biological material. Upconverting NPs (UCNP) are widely used in various biological applications such as labeling, imaging [1] and photothermal therapy. UCNP can be doped simultaneously with sensitizer ions (Yb^{3+}) and activator ions (Er^{3+}). The sensitizer ions absorb the NIR photons and then transfer the energy to the activator ions. Energy transfer excites the activators to their higher excited states and ultimately leads to the radiation of higher energy photons. Additional doping with neodymium ions (Nd^{3+}) allows for the excitation of UCNP (cross-transfer between Er^{3+} -Yb^{3+} -Nd^{3+} ions) with a laser with a wavelength of 808 nm, which significantly reduces the effect of overheating of biological samples. The water absorption coefficient value at 980 nm is 0.48 cm^{-1}, while the value at 808 nm is much lower and amounts to 0.02 cm^{-1} [2][3].

The hybrid core/shell Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18%Yb^{3+}, 2.5%Mg^{2+}, x% Nd^{3+} NPs doped with different concentration of neodymium ions (x = 0%, 0.5%, 0.75%, 1%, 2%, 4%) were synthesized by co-precipitation method. The NPs were characterized using X-ray diffraction, transmission electron microscopy, scanning electron microscopy, energy dispersive x-ray spectroscopy, confocal microscopy and photoluminescence. Fe_3O_4 (core) nanoparticles were 13 nm in size. Whereas the hybrid core/shell NPs had sizes ranging from 220 nm to 641 nm. The shell thicknesses were 72, 80 and 121 nm for 0.5%, 0.75% and 1% of Nd^{3+} concentration, respectively.

The obtained core/shell NPs had a cubic Gd_2O_3 crystal structure (symmetry group Ia-3). The UCNP efficiency properties and magnetic properties of the hybrid NPs were investigated. The intensity of the upconversion emission was increased by the addition of Nd^{3+} ions and achieved maximum value at a neodymium ion concentration of 0.5% for the 980 nm excitation and 1% Nd^{3+} for the 808 nm excitation. The hybrid core/shell nanoparticles were paramagnetic. At room temperature, the magnetization was 0.02 emu/g for NPs with an average shell thickness of 72 nm. The toxicity of the Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18%Yb^{3+}, 2.5%Mg^{2+}, 0.5% Nd^{3+} NPs was investigated, in the presence of HeLa tumor cells for 24h. The NPs are non-toxic up to a concentration of 1000 μg/ml and penetrate into cells in the process of endocytosis, which has been confirmed by confocal microscope studies.

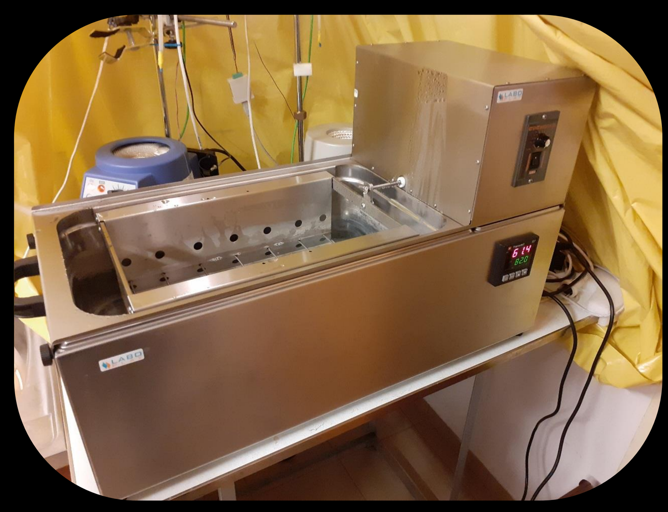
1. I. Kamińska et al, Nanotechnology 2021, 32, 245705 (13pp).
2. Liu et al, Adv. Mater. 2017, 29(18), 1605434.
3. Gao et al, ACS Appl. Energy Mater. 2021, 4, 2999−3007.
4. Ge et al., CrystEngComm, 2015,17, 5702-5709 .

NANOPARTICLES SYNTHESIS

Synthesis of Fe_3O_4 magnetic NPs

$FeCl_3 \cdot 6H_2O$
$FeCl_2 \cdot 4H_2O$
40 ml H_2O

argon atmosphere
5 ml ammonium hydroxide 28-30%
1.3 g poly(ethylene glycol) PEG 4600 in 5 ml H_2O
at 90°C for 1h
black precipitate
washed several times in H_2O and ethanol
centrifugation parameters: 6000 rpm, 15°C, 15 minutes
dried in laboratory oven overnight



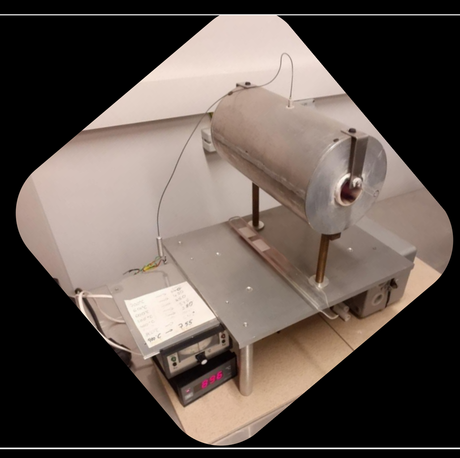
Synthesis of the hybrid $Fe_3O_4/Gd_2O_3:Er^{3+}, Yb^{3+}, Mg^{2+}, Nd^{3+}$ NPs

30 mg Fe_3O_4
100 ml H_2O
Sonicated for 30 minutes

HOMOGENOUS PRECIPITATION METHOD

Oxidants	Reducer
$Gd(NO_3)_3 \cdot 6 H_2O$	$CO(NH_2)_2$
$Nd(NO_3)_3 \cdot 6 H_2O$	
$Mg(NO_3)_2 \cdot 6H_2O$	
$Er(NO_3)_3 \cdot 5 H_2O$	
$Yb(NO_3)_3 \cdot 5 H_2O$	

at 90°C for 2h
washed five times in deionized water
centrifugation parameters: 6000 rpm, 15°C, 15 minutes
white-brown precipitate was dried in a laboratory oven overnight
calcined in an oven, in air for 2h at 700°C



NANOPARTICLE SIZE BY SCANNING ELECTRON MICROSCOPY

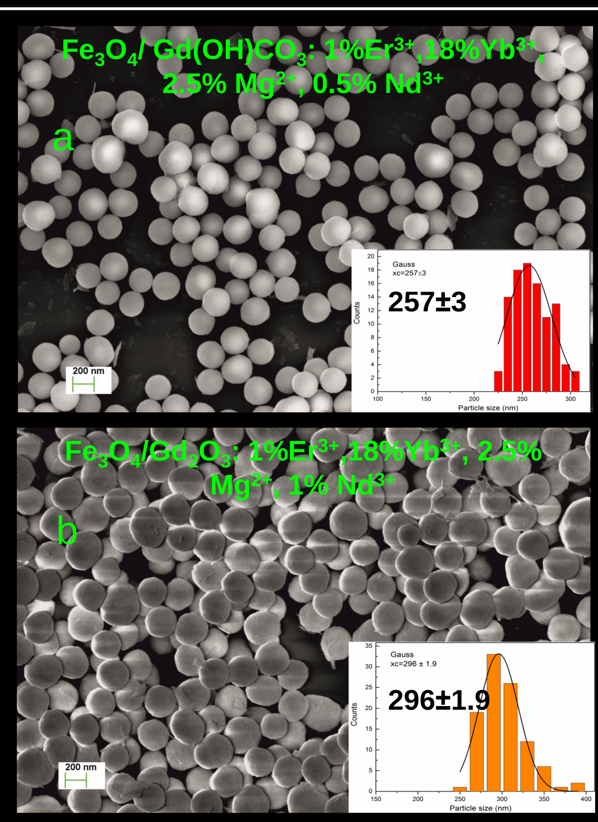


Figure 5. SEM images of the a) $Fe_3O_4/Gd(OH)CO_3$: 1%Er^{3+},18%Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} and b) Fe_3O_4/Gd_2O_3: 1%Er^{3+},18%Yb^{3+}, 2.5% Mg^{2+}, 1% Nd^{3+} NPs (calcined in air, for 2h at 700°C). Inserts: Size distribution histograms of the NPs.

TRANSMISSION ELECTRON MICROSCOPY

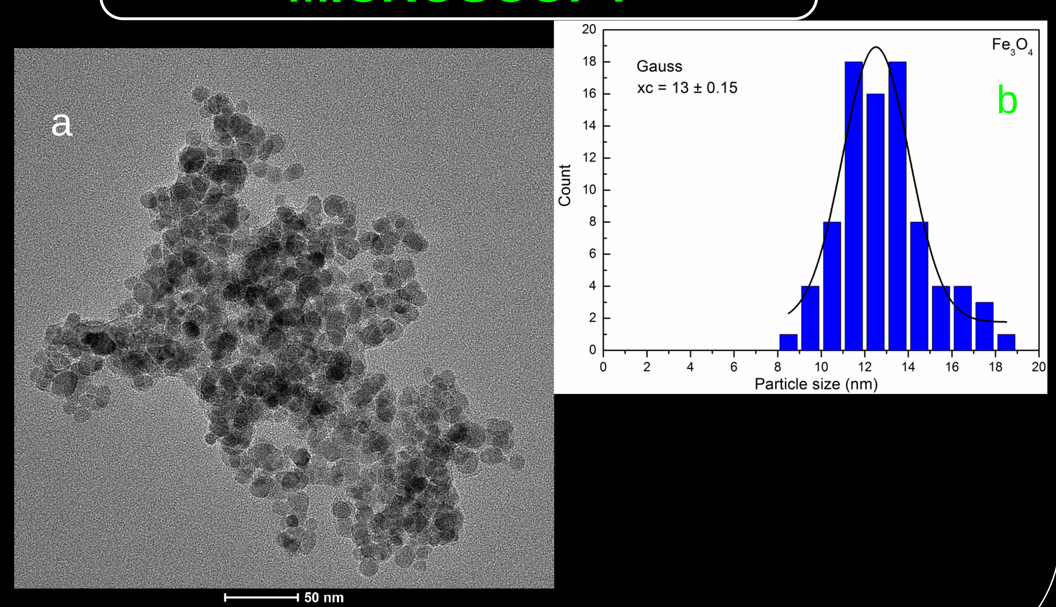


Figure 1. a) TEM image of Fe_3O_4 NPs b) Size distribution histogram of the NPs.

UPCONVERSION MECHANISM

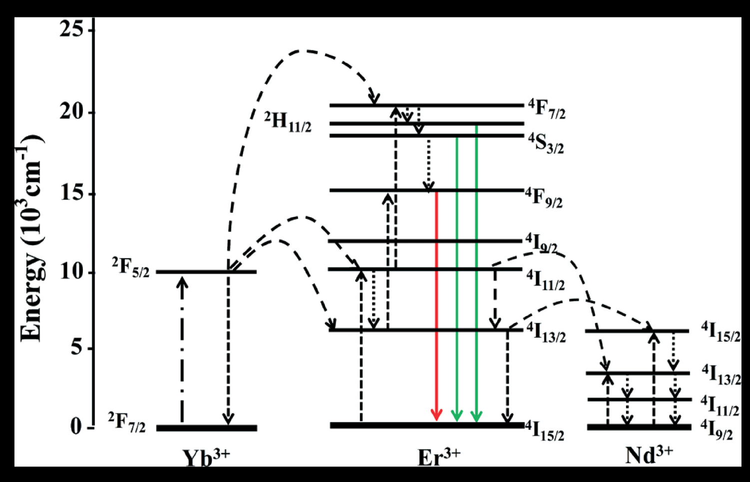
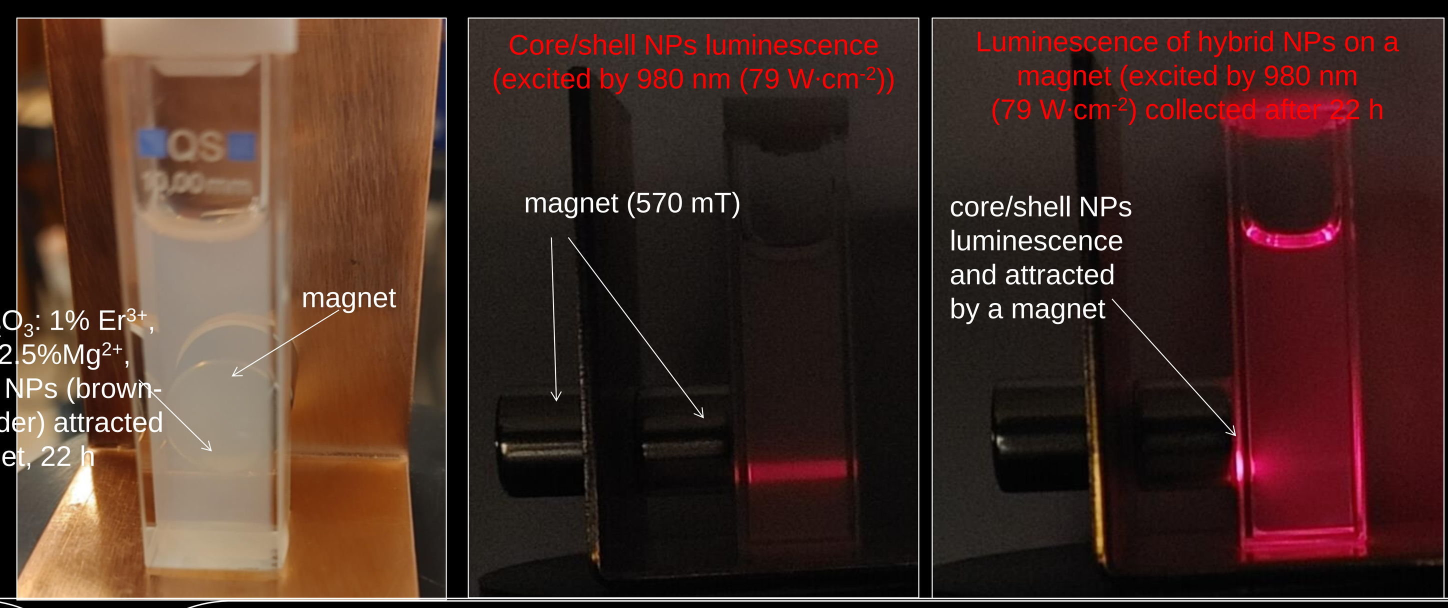


Figure 3. The proposed energy transfer mechanisms under 980 nm CW laser excitation in Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18%Yb^{3+}, 2.5%Mg^{2+}, 0.5% Nd^{3+} nanoparticles. The dashed-dotted, dashed, dotted, and full arrows represent photon excitation, energy transfer, multiphonon relaxation, and emission process, respectively [4].

Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18%Yb^{3+}, 2.5%Mg^{2+}, 0.5% Nd^{3+} NPs ATTRACTED BY A MAGNET (NdFeB)



ENERGY DISPERSIVE X-RAY SPECTROSCOPY

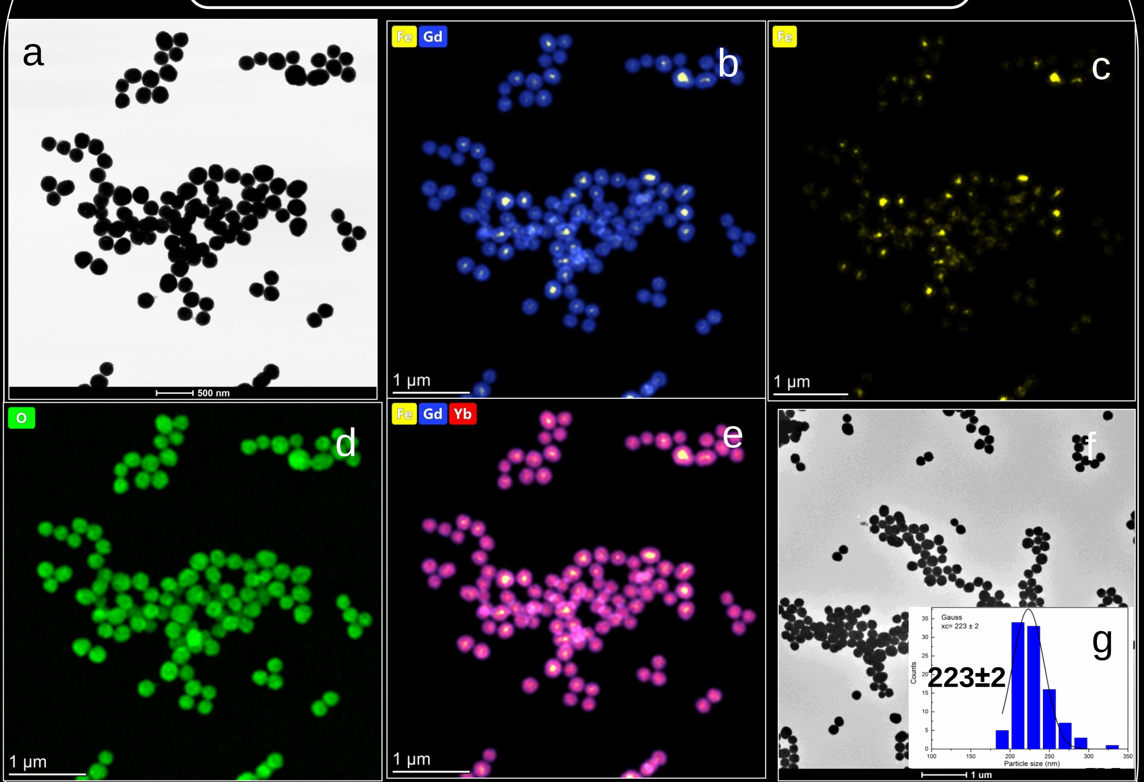


Figure 2. TEM images of Fe_3O_4/Gd_2O_3:1% Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} nanoparticles a) in bright field. The distribution maps of the elements of the NPs b) Fe and Gd c) Fe d) O e) Fe, Gd and Yb. f) TEM image of the nanoparticles. g) Size distribution histogram of the NPs.

X-RAY DIFFRACTION

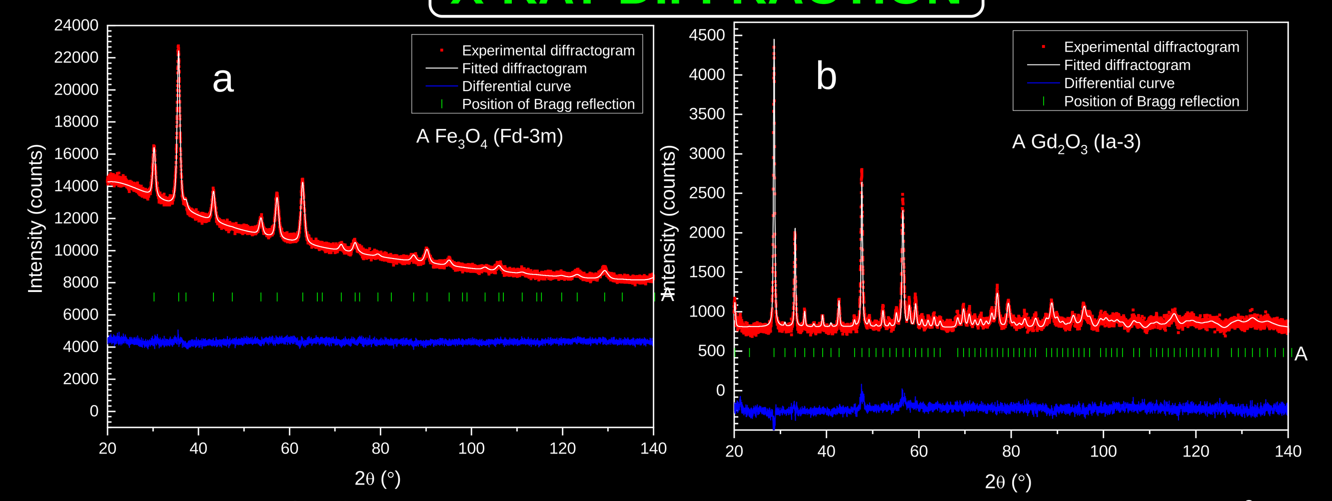


Figure 4. Experimental diffractograms measured for a) Fe_3O_4 NPs and b) Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} NPs and matched by the means of the Rietveld method theoretical diffractograms. Symbols: (•) experimental and (–) fitted diffractograms, (–) differential curve and (|) positions of Bragg reflections (coming from Fe_3O_4 and Gd_2O_3 phase, respectively).

PHOTOLUMINESCENCE

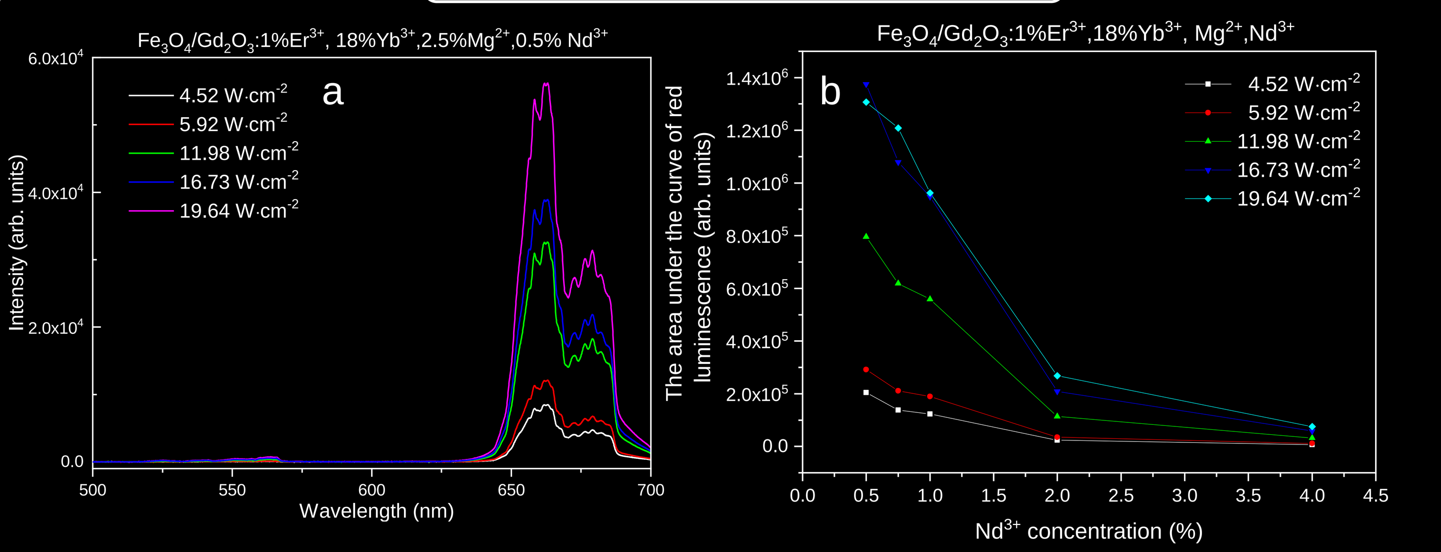


Figure 6. a) The photoluminescence spectra of the Fe_3O_4/Gd_2O_3:1%Er^{3+},18%Yb^{3+},2.5%Mg^{2+},0.5%Nd^{3+}NPs. The NPs were excited with five power densities of a semiconductor laser with a wavelength of 980 nm. b) Area under the curve of red luminescence as a function of neodymium ion concentration for the NPs.

MAGNETIC PROPERTIES

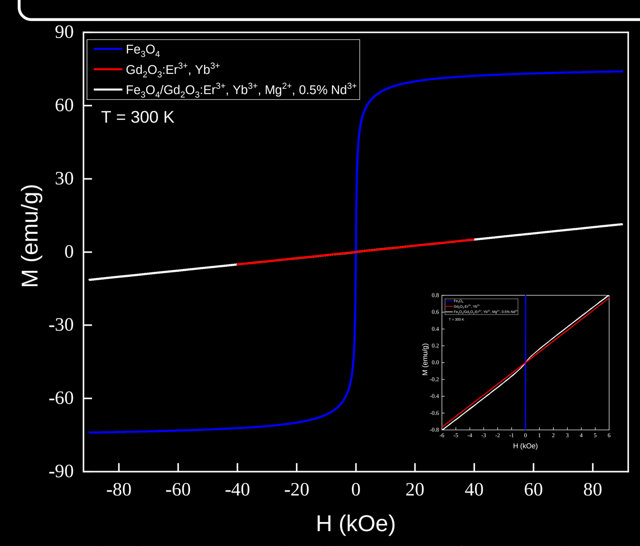


Figure 9. Magnetization curves for three samples: Fe_3O_4, Gd_2O_3: 1%Er^{3+}, 18% Yb^{3+} and Gd_2O_3: 1%Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} NPs measured at 300 K.

TOXICITY TEST OF NPs

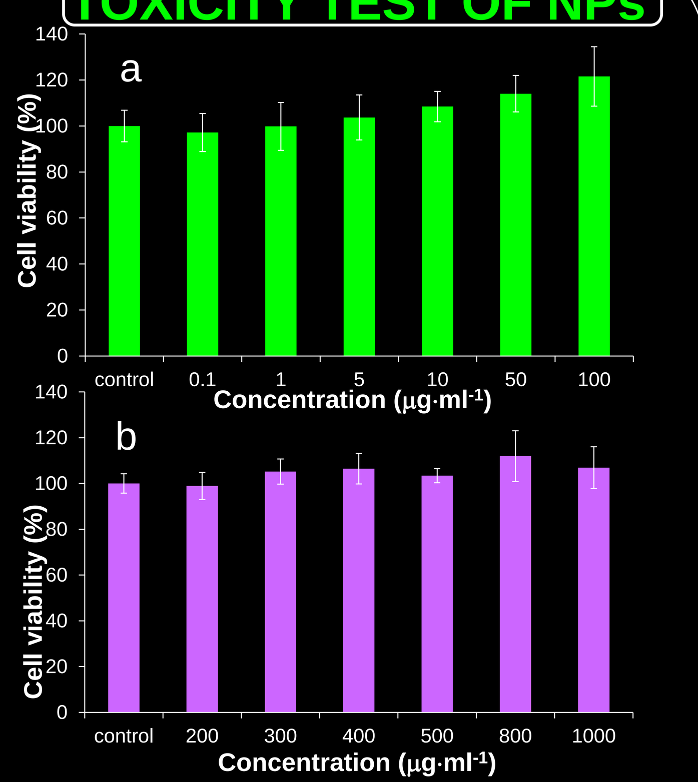


Figure 7. Cell viability of HeLa cells after 24h incubation with twelve concentrations of Fe_3O_4/Gd_2O_3:1% Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} NPs as determined by PrestoBlue assay.

CELL CULTURE AND CONFOCAL *IN VITRO* IMAGING

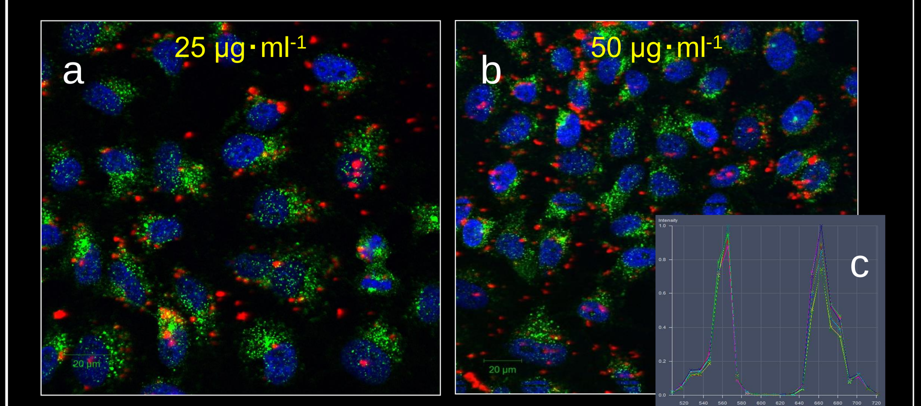


Figure 8. Confocal images of HeLa cancer cells after 24 h incubation in a solution with a) 25 μg·ml^{-1} and b) 50 μg·ml^{-1} of Fe_3O_4/Gd_2O_3:1% Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} NPs. The NPs were excited with a 980 nm femtosecond laser (observed as red spots and d) colorful indicators). The HeLa cells were marked using immunofluorescence method. Antibodies conjugated with AlexaFluor 488 dye (excited by 488 nm argon laser) were attached to the lysosomes. The signal was collected in the range from 496 to 570 nm (observed as a green regions). Nuclei stained with Hoechst 33 342 dye were excited with a wavelength of 705 nm (blue color). The signal was collected in the range from 423 to 475 nm. The images are a superposition of NPs luminescence, marked cells fluorescence, and nuclei fluorescence. c) The spectra of NPs were excited by a femtosecond laser at a wavelength of 980 nm and average laser power of 10%.

Acknowledgements

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CONCLUSIONS

1. Optical-magnetic core/shell NPs were synthesized by homogeneous precipitation synthesis.
2. The nanoparticles show emission in the visible region (562 nm and 662 nm) when excited with a semiconductor laser with a wavelength of 980 nm and 808 nm.
3. The hybrid nanoparticles range in size from 220 to 641 nm, depending on the neodymium ion concentration.
4. The produced Fe_3O_4 nanoparticles have a cubic crystal structure (symmetry group Fd-3m). Whereas the obtained Fe_3O_4/Gd_2O_3: 1% Er^{3+}, 18% Yb^{3+}, 2.5% Mg^{2+}, 0.5% Nd^{3+} nanoparticles have a cubic Gd_2O_3 crystal structure (symmetry group Ia-3).
5. Nanoparticles contain elements such as iron (Fe) and oxygen (O). To confirm the presence of elements such as iron (Fe), gadolinium (Gd), oxygen (O) and ytterbium (Yb) EDX maps were made.
6. The core/shell nanoparticles are paramagnetic.
7. For the magnetic field strength H=100 Oe, the magnetization for Fe_3O_4 NPs is 12 emu/g, for Gd_2O_3: 1%Er^{3+} , 18% Yb^{3+} is 0.013 emu/g, and for the hybrid core/shell NPs is 0.02 emu/g.
8. Nanoparticles penetrate into HeLa cancer cells by endocytosis, which was confirmed by confocal microscopy studies.
9. The core/shell nanoparticles are non-toxic to cells up to a concentration of 1000 ug/ml, which was confirmed by studies using the PrestoBlue test.