Label-free iron oxide nanoparticles as multimodal contrast agents in cells using multi-photon and magnetic resonance imaging.

Hendrik Reynders¹, Indra Van Zundert¹,^a Rui Silva^{1;2;5}, Bram Carlier⁵, Olivier Deschaume³, Carmen Bartic³, Susana Rocha¹, Sergey Basov⁴, Margriet J. Van Bael⁴, Uwe Himmelreich⁵, Thierry Verbiest¹ and Ana Zamora^{1,5}

¹ Molecular Imaging and Photonics, KU Leuven, Leuven, Belgium;

² Engineering Department, Oporto University, Porto, Portugal;

³ Laboratory for Soft Matter and Biophysics, KU Leuven, Leuven, Belgium;

⁴ Department of Quantum Solid State Physics, KU Leuven, Leuven, Belgium;

⁵ Department of Imaging and Pathology, KU Leuven, Leuven, Belgium.

Corresponding author: Ana Zamora

E-mail: anamaria.zamoramartinez@kuleuven.be

ORCID: 0000-0001-7013-8900



Figure S1. UV/Vis absorption spectrum of PEG-coated IONPs in dH₂O (0.01 mg of Fe/mL).



Figure S2. One-photon emission spectra (left) of OA-IONPs dispersed in heptane with an average diameter of a) 7 nm, b) 11 nm and c) 15 nm at different excitation wavelengths, and their respective TEM-images (right). The iron concentration for all samples was kept at 0.1 mg/mL.

0.02



Figure S3. One-photon emission spectra of OA-IONPs dispersed in heptane with a concentration of a) 0.1 mg/mL, b) 0.5 mg/mL and c) 1 mg/mL at different excitation wavelengths.



Figure S4. Plot of the multi-photon emission intensity of PEG-coated IONPs in function of the incident laser power. The quadratic dependence confirms that the multi-photon emission is a two-photon process.



Figure S5. Cell uptake in A549 cells after 24 h incubation. Samples with 2 cell counts of 20000 and 50000 cells and with different PEG-coated IONPs concentrations added from 0 to 250 μ g/mL were digested and the iron content was quantified with ICP-MS.



Figure S6. XTT cytotoxicity assay of a) A549 cells, b) BEAS-2B cells and c) GRX cells incubated with PEGcoated IONPs at different concentrations for 24 h, 48 h and 72 h, respectively). Data was collected from two independent experiments with three replicates per sample and analyzed with Two-Way ANOVA with Bonferroni post-Hoc test: Interaction (P>0.05) and One-Way ANOVA with Dunnet's post-Hoc: (* P<0.05 and difference >10%)



Figure S7. Cell ratio over control (cells without NPs treatment) after the incubation of A549 cells with PEGcoated IONPs at different concentrations for different durations. The decrease of cell ratio when increasing the concentration of PEG-IONPs indicates that cell replication stopped and therefore that PEG-IONPs present some toxicity at high concentration.



Figure S8. T2 maps of A549 cells labeled with PEG-coated IONPs. a) Eppendorfs 1 to 4 (50 cells/mL) and 5 to 8 (100 cells/mL) and Fe concentration of 0 (1 and 5), 50 (2 and 6), 100 (3 and 7) and 250 ug/mL (4 and 8); b) Eppendorfs 1 to 4 (250 cells/mL) and 5 to 8 (400 cells/mL) and Fe concentration of 0 (1 and 5), 50 (2 and 6), 100 (3 and 7) and 250 ug/mL (4 and 8).