# Supporting Information

## Surgery-Guided Removal Of Ovarian Cancer Using Up-Converting Nanoparticles

Christopher B. Marotta[1a] *, Tom Haber[2a], Jacob M. Berlin[2b], Robert H. Grubbs[1b]

[1]Department of Chemistry and Chemical Engineering at California Institute of Technology, 1200 E. California Blvd, Pasadena, California, 91125, United States

[2]Department of Molecular Medicine at City of Hope, 1500 East Duarte Road, Duarte, California 91010, United States.

[a]Co-Authorship

[b]Co-Principle Investigators

*Corresponding Author: Christopher B. Marotta (marotc10@gmail.com)

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## ***Experimental Methods***

### Synthesis of TFA Salt Precursors

Single element oxides (ytterbium or neodymium) and oxide mixtures (ytterbium:holomiun 99:1 molar ratio) were weighed out to a total of 1 mmol and placed in a 100 mL flask. 25 mL of trifluoroacetic acid and 25 mL of DI water were added and brought to reflux for 2 hours. The solution was filtered hot to remove any undissolved material. Excess water/trifluoroacetic acid was removed with a rotary evaporator. The recovered lanthanide TFA salt was further dried under high vacuum at 50 °C for at least 4 hours. 1 mmol of material yields ~ 4 grams of lanthanide TFA salt and can be stored in air at room temperature for use later.

### UCNP Syntheses

*Synthesis – $NaYF_4$@$NaYbF_4$:Ho(1%)@$NaYF_4$* ***(Yb-Ho-1)***

The synthesis of UCNPs for this project was modified from previously reported protocols.[1–10] Lanthanide trifluoroacetate (TFA) salts are synthesized and used as precursors for the thermolysis approach. This approach provides synthetic control of the particle compositions and a modular method for facile layering construction. A high degree of complexity is easily attainable.

The core mixture contained 1 mmol of yttrium TFA and 4 mmol of sodium TFA in 8.5 mL oleic acid and 8.5 mL of 1-octadecene. The 1st shell mixture contained 0.5 mmol of ytterbium:holmium (99:1) TFA and 2 mmol of sodium TFA in 4 mL oleic acid and 4 mL of 1-octadecene. The 2nd shell mixture contained 1 mmol of yttrium TFA and 4 mmol of sodium TFA in 7 mL oleic acid and 7 mL of 1-octadecene. Each flask was heated to 100 °C under vacuum for 30-60 min to remove oxygen and water and form their corresponding oleate complexes (all solids should be dissolved). All flasks were back filled with argon and the shells were kept at 50 °C. The core was heated to 320 °C at a rate of ~10 °C/min and maintained at that temperature for 70 min using a thermocouple and the Apollo temperature controller (J-KEM Scientific). All of the 1st shell was added slowly as to not drop the overall reaction flask below 300 °C. The reaction was incubated for 45 min once all the solution had been added. The 2nd shell was added to the main reaction flask in an identical fashion to the 1st shell. The reaction was removed from the heating mantel and cooled to room temperature naturally (~30 min). The cooled solution was transferred to two 50 mL Falcon tubes in equal volumes. An equal volume ethanol (19.5 mL) was added to each tube to precipitate the UCNPs. Particles were collected via centrifuge for 15 min and appeared as

an off-white pellet. The UCNPs were then dispersed in 25 mL of ethanol and collected via centrifuge for 5 min. This was repeated identically a second time. The ethanol washes were done to remove residual compounds from the original reaction. The final pellet of UCNPs was dried using a stream of argon for 30 min. The pellet (white) was weighed and dissolved in either chloroform or cyclohexane at a concentration of ~20 mg/mL. The collected UCNP's (**Yb-Ho-1**) outer layer is coated with oleic acid as a result of the synthesis and can be stored in solution or as the dried pellet for later use.

*Synthesis – NaY:Yb:Ho(10:89:1)$F_4$@NaYb:Y(9:1)$F_4$@NaNd:Y(9:1)$F_4$@$NaYF_4$ (**Yb-Ho-2**)*

Synthesis of a multi-shell UCNP follows a near identical protocol as the previous synthesis of **Yb-Ho-1** but with an extra shell addition and change of compositions and ratios for each layer.

The core mixture contained 0.1 mmol of yttrium TFA, 0.9 mmol of ytterbium:holmium (99:1) TFA, and 4 mmol of sodium TFA in 11 mL oleic acid and 11 mL of 1-octadence. The 1st shell mixture contained 0.05 mmol of yttrium TFA, 0.45 mmol of ytterbium TFA, and 2 mmol of sodium TFA in 4 mL oleic acid and 4 mL of 1-octadecene. The 2nd shell mixture contained 0.05 mmol of yttrium TFA, 0.45 mmol of neodymium TFA, and 2 mmol of sodium TFA in 4 mL oleic acid and 4 mL of 1-octadecene. The 3rd shell mixture contained 0.5 mmol of yttrium TFA and 2 mmol of sodium TFA in 4 mL oleic acid and 4 mL of 1-octadence. Each flask was heated to 100 °C under vacuum for 30-60 min to remove oxygen and water and form their corresponding oleate complexes (all solids should be dissolved). All were back filled with argon and the shells were kept at 50 °C. The core was heated to 320 °C at a rate of ~10 °C/min and maintained at that temperature for 60 min. All of the 1st shell was added slowly as to not drop the overall reaction flask below 300 °C. The reaction was incubated for 45 min once all the solution had been added. The 2nd shell was added to the main reaction flask in an identical fashion to the 1st shell. Then the 3rd shell was added to the main reaction flask in an identical fashion to the 1st shell. The reaction was removed from the heating mantel and cooled to room temperature naturally (~30 min). The entire cooled solution was transferred to two 50 mL Falcon tubes in equal volumes (some material precipitated in the flask due to the size of the particles). An equal volume of ethanol (23 mL) was added to each tube to precipitate the UCNPs which were collected via centrifugation for 15 min and appeared as an off-white pellet. The UCNPs were then dispersed in 25 mL of ethanol and collected via centrifuge for 5 min. This was repeated identically a second time. The ethanol washes were done to remove residual compounds from the original reaction. The final pellet of UCNPs was dried using a stream of argon for 30 min. The pellet (white) was weighed and dissolved in either chloroform or cyclohexane at a concentration of

~20 mg/mL. Again, the collected UCNP's (**Yb-Ho-2**) outer layer is coated with oleic acid as a result of the above synthesis and can be stored in solution or as the dried pellet for later use.

**Silica Coating Procedure**

The following silica coating procedure was modified from a previously reported protocol[11,12] and used to silica coat the above synthesized UCNPs. 15.5 mL of cyclohexane, 3.2 mL of 1-hexanol, 4 mL of Triton X-100, and 0.68 mL DI water were vigorously stirred for 15 min. Then, ~40 mg of UCNPs (2 mL of dissolved stock in cyclohexane) were added and stirred an additional 5 min at an RPM of 600. TEOS (20 μL) was added and the mixture was allowed to stir for 2 hours. 200 μL of ammonium hydroxide were added and stirred at room temperature for 48 hours.

The reaction was transferred to a 50 mL Falcon tube. 10 mL ethanol was used to rinse the flask and transfer the rest to the Falcon tube. 10 mL of acetone were added to precipitate the silica coated UCNPs, which were collected by centrifugation. UCNPs were resuspended in 20 mL of a 1:1 water:ethanol solution and recollected by centrifugation. This washing process was done 2-3 times. The final pellet of UCNPs was dried using a stream of argon for 30 min. The pellet (white) was weighed and dispersed in DI water at a concentration of ~10 mg/mL. This procedure produced the silica coated variants of **Yb-Ho-1** and **Yb-Ho-2**. These will be referred to as the following for clarity:

**Yb-Ho-$SiO_2$-1** ($NaYF_4$@$NaYbF_4$:Ho(1%)@$NaYF_4$@$SiO_2$**)**,
**Yb-Ho-$SiO_2$-2** (NaY:Yb:Ho(10:89:1)$F_4$@NaYb:Y(9:1)$F_4$@NaNd:Y(9:1)$F_4$@$NaYF_4$@$SiO_2$)

**Injection, Dissection, and Imaging**

*Cell Culture*

All cells were cultured and maintained at 37 °C in a humidified incubator containing 5% $CO_2$. OVCAR8-GFP cells were cultured in Dulbecco's Modifed Eagle's Medium (DMEM; Invitrogen, CA, USA) supplemented with 10% fetal bovine serum (Gemini Bio, CA, USA), 1% L-glutamine (Invitrogen) and 1% penicillin–streptomycin (Invitrogen). The cells were stably transduced with eGFP. When the cells reached 80% confluency, they were passaged using a 0.25% trypsin/EDTA solution (Invitrogen); media was changed every 2–3 days.

The synthesized UCNP@Silica particles were tested in the metastatic mouse model of ovarian cancer. Mice were inoculated with OVCAR8.eGFP cells via intraperitoneal (IP) injection. GFP expressing

cancer was used as a way to verify that the cells accumulating the UCNPs are cancerous and not healthy tissue. After 3 weeks, a dose of UCNPs of $1.37*10^{10}$ nanoparticles was administered by an IP injected. Four days after the UCNP@Silica injection, the mice were euthanized and all of the abdominal organs were removed for imaging. Whole organs were imaged using a whole-body imaging system and a dissecting macroscope. The GFP molecules are excited with ~400 nm light. The UCNPs were excited with a 980 nm laser diode. The presence of UCNP uptake was confirmed by green emission when the samples are exposed only to a 980 nm light source (false colored as red).

For human samples of ovarian tumors and healthy tissue, a similar procedure was used. Freshly excised samples were incubated for 4 days in a buffer solution containing $1.37*10^{10}$ nanoparticles of UCNP@silica particles and then washed away. They were imaged using a dissecting macroscope with the 980 nm light exposure. The presence of UCNP uptake was confirmed by green emission when the samples were exposed only to a 980 nm light source (false colored as red).

***Characterization of UCNPs***

Emission spectra were collected on a Kymera 193i spectrograph (Oxford Instruments, Andor Solis). A 1 W, 980 nm laser was used as the excitation source (MDL-III-980-1W from Changchun New Industries (CNI laser) Optoelectronics Tech. Co., Ltd). Transmission electron microscopy (TEM) images were obtained from a FEI TF30ST transmission electron microscope. Scanning electron microscopy (SEM) images and energy dispersive spectrometer (EDS) data were collected from a ZEISS 1150VP FESEM with an Oxford X-Max SDD X-ray EDS system. The concentration of nanoparticles and their size (mean diameter) were measured using the NanoSight NS300 and analyzed with the nanoparticles tracking analysis software (Malvern NanoSight NS300 instrument, NTA software). Measurements were obtained by performing 3 runs of 60 seconds each, sample flow rate was controlled and kept constant (speed = 30) during the acquisition using a syringe pump.

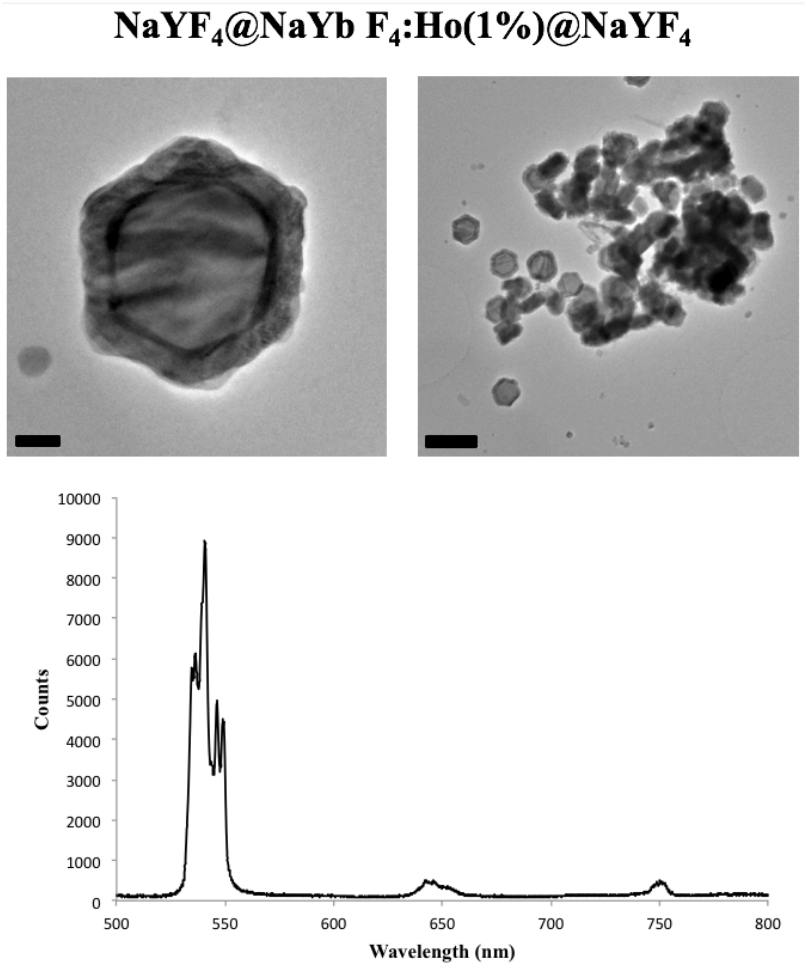


**Figure S1**: *Top row* – TEM images of as synthesized oleic acid-coated UCNPs (**Yb-Ho-1**). Left image scale bar = 50 nm. Right image scale bar = 0.5 μm. Large hexagonal shaped UCNPs are obtained and discrete layers can be seen in the particles. The coloring is most likely due to the density difference between the layers. *Bottom* – emission spectra of oleic acid coated UCNPs using a 980 nm excitation with a power density of 2.5 W/cm$^2$. A near single large emission peak is observed at ~545 nm, which is responsible for the bright green color produced by the particles.

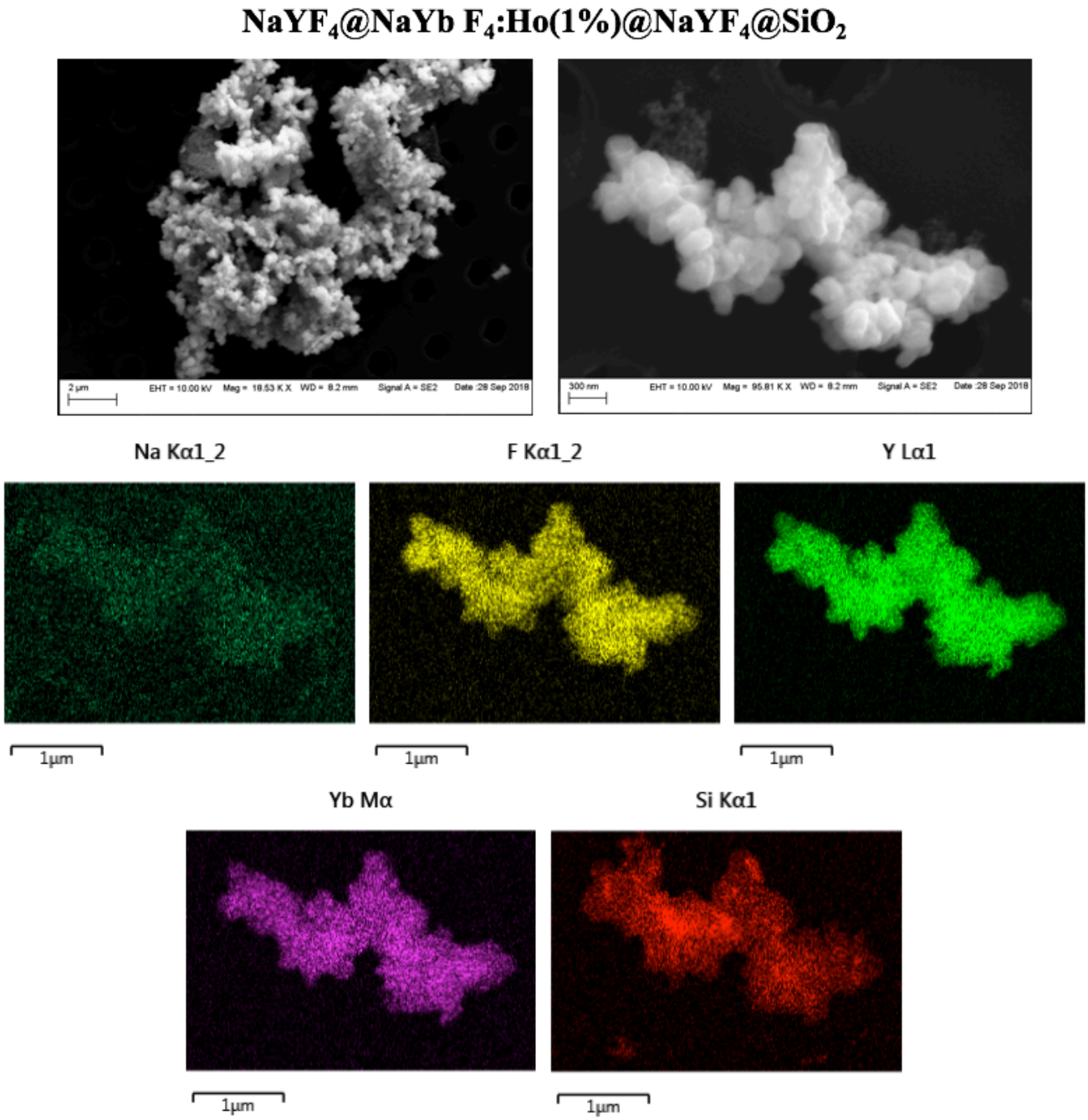


**Figure S2**: The top two images are SEM images of the silica-coated UCNPs (**Yb-Ho-SiO$_2$-1**). The bottom images are the individual elemental analyses from the EDS instrument. The elements x-ray fluorescence are seen: sodium (Na), fluorine (F), yttrium (Y), ytterbium (Yb), and silicon (Si). Holmium (Ho) is in too small of concentration to be detected by this technique. Most importantly, this analysis shows the presence of silicon associated with the particle, which corroborates successful silica coating.

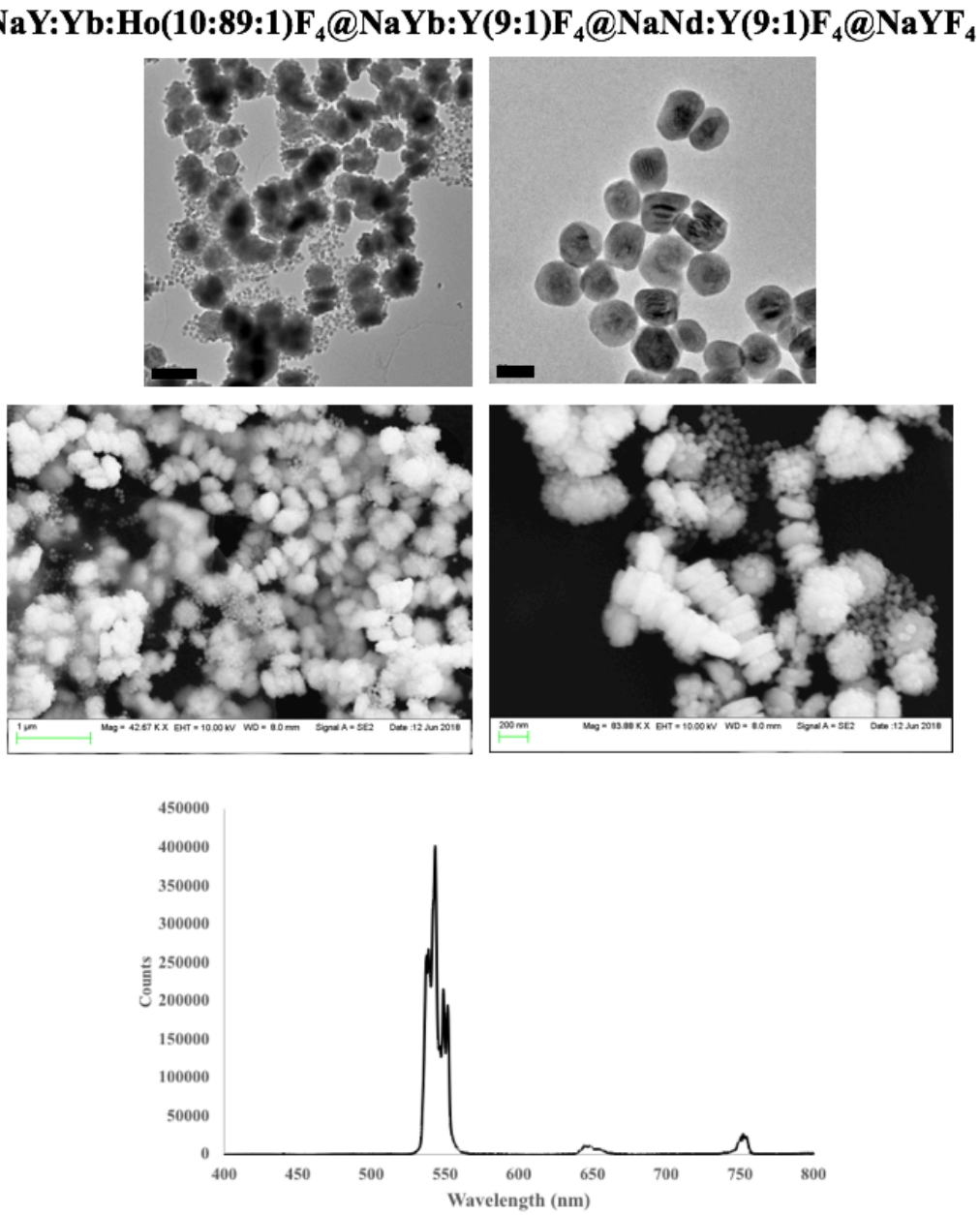


**Figure S3**: *Top row* – TEM images of as synthesized oleic acid-coated UCNPs (**Yb-Ho-2**). Left image scale bar = 0.5 μm. Right image scale bar = 50 nm. *Middle row* – SEM images of the same UCNPs. Left image scale bar = 1 μm. Right image scale bar = 200 nm. Large (>150 nm) and small (<50 nm) UCNPs are observed. The small particles look similar to other synthesized particles ($NaNdF_4$@$NaYF_4$) not shown here. This could indicate a non-homogenous layer growth of the final two shell additions. The particles are large and could crash out of solution despite the elevated temperatures. *Bottom* – emission spectra of oleic acid coated UCNPs using a 980 nm excitation with a power density of 2.5 W/cm$^2$. A near single large emission peak is observed at ~545 nm, which is responsible for the bright green color produced by the particles.

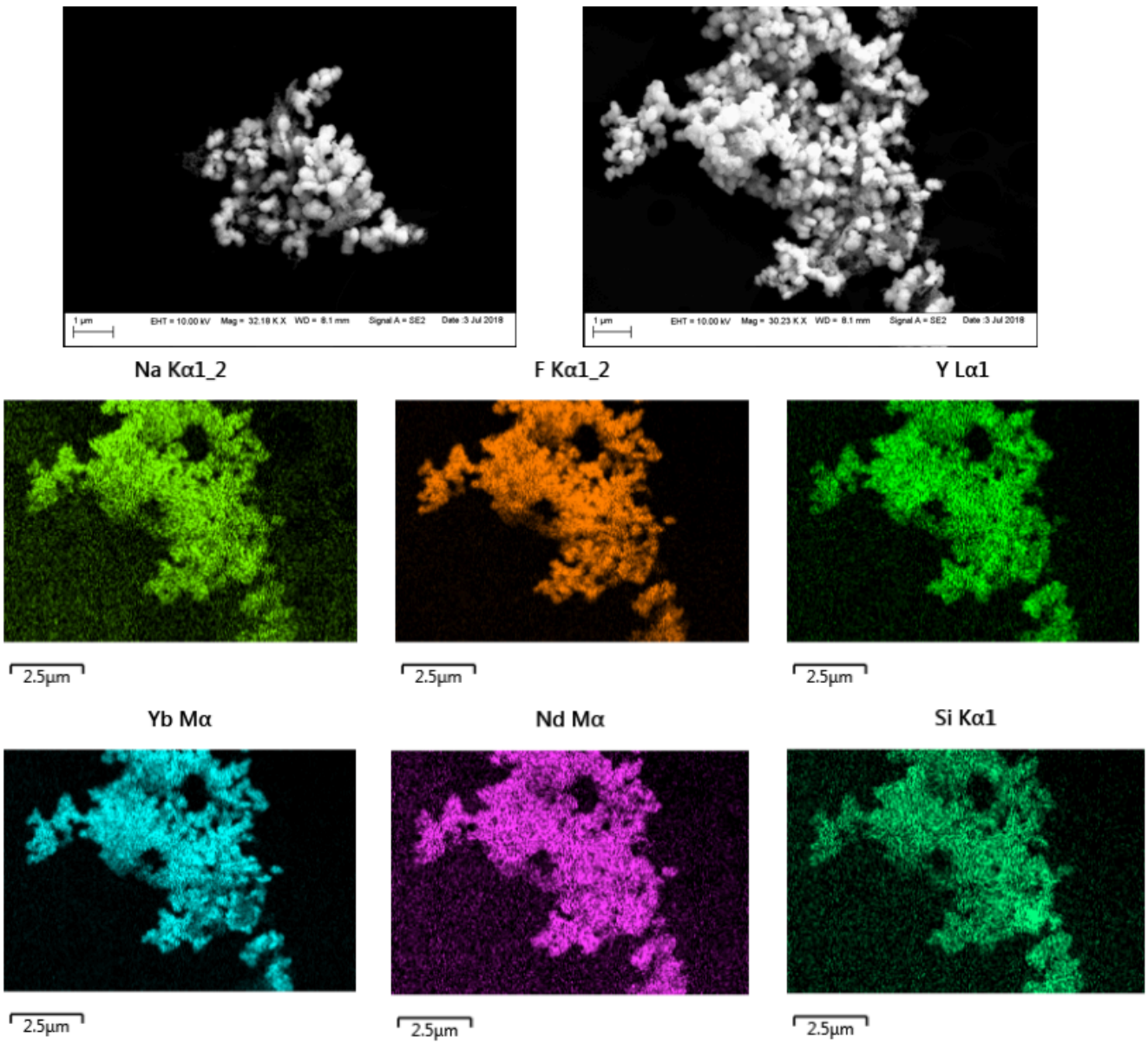


**Figure S4**: The top two images are SEM images of the silica-coated UCNPs (**Yb-Ho-SiO$_2$-2**). The bottom images are the individual elemental analyses from the EDS instrument coupled to the machine. The elements x-ray fluorescence are seen: sodium (Na), fluorine (F), yttrium (Y), ytterbium (Yb), neodymium (Nd), and silicon (Si). Holmium (Ho) is in too small of concentration to be detected by this technique. Most importantly, this analysis shows the presence of silicon associated with the particle, which corroborates successful silica coating.

***Expanded Imaging Studies***

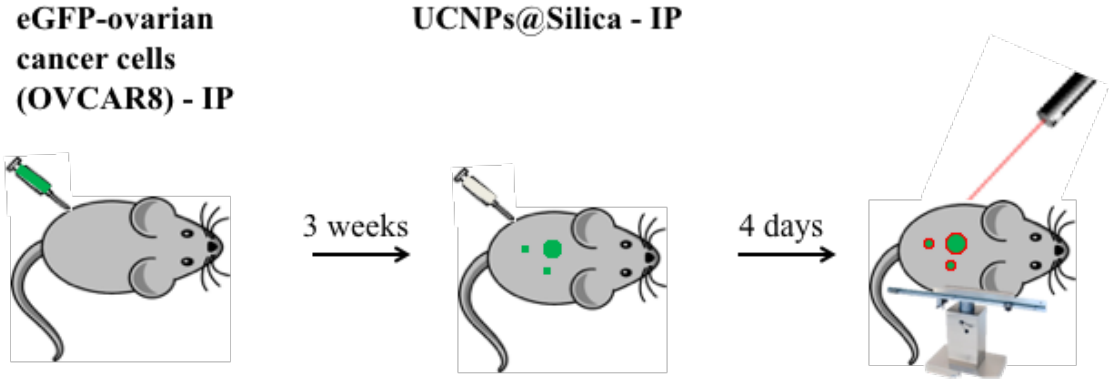


**Figure S5**: Experimental outline for mouse injection and imaging. An intraperitoneal injection (IP injection) of ovarian cancer expressing GFP cells is performed and allowed to incubate for 3 weeks. An IP injection of silica coated UCNPs is performed and allowed to incubate for 4 days. The mouse is then sacrificed and the organs are imaged using light sources that match the GFP (~400 nm) and UCNPs (980 nm). Images are recorded with a camera attached to the dissecting macroscope.

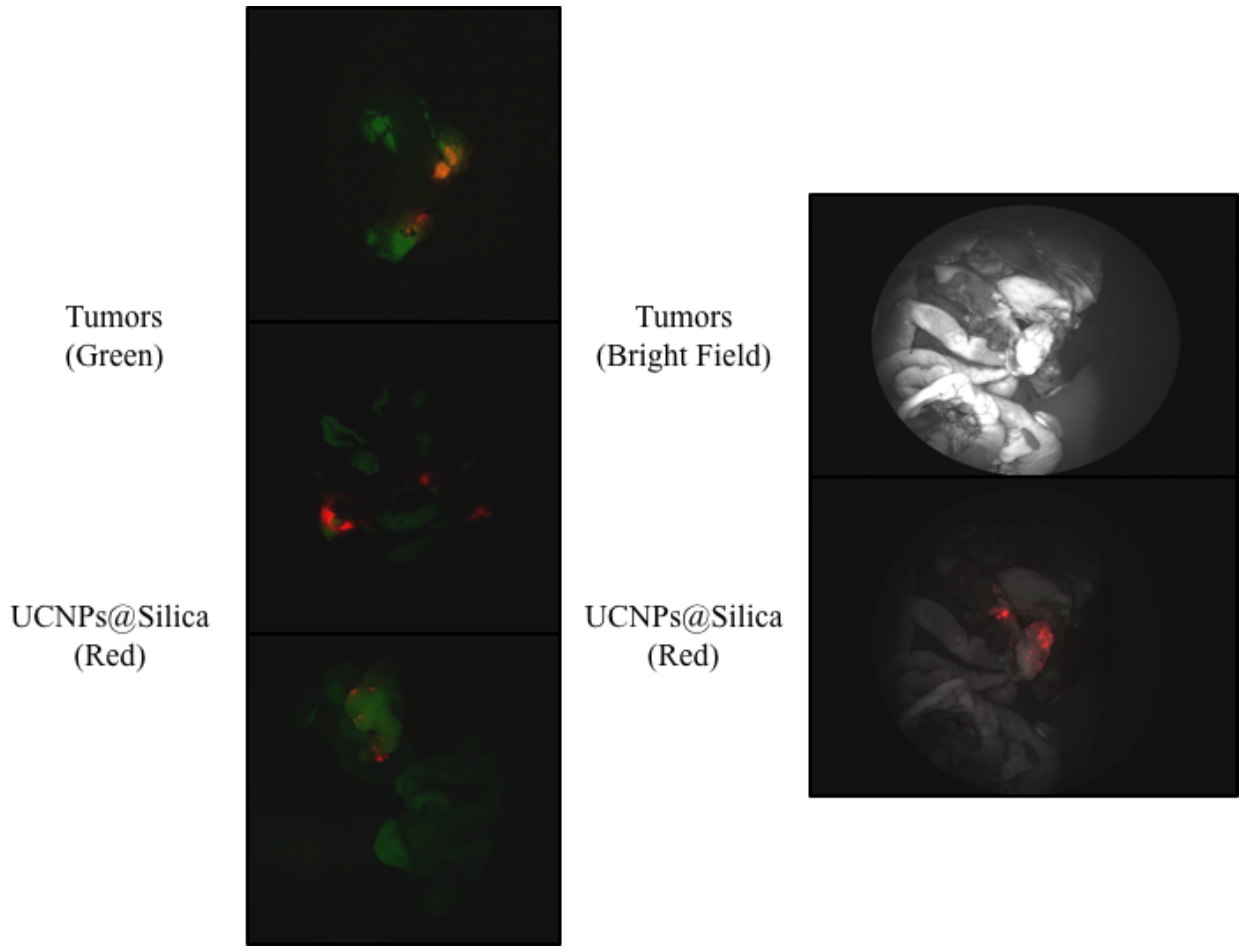


**Figure S6**: Left - merged images of the UCNP and GFP fluorescence from excised organs. Right – merged images of the UCNP fluorescence and bright field image. **Yb-Ho-$SiO_2$-1** were injected and emit green (545 nm). The fluorescence of the UCNPS was falsely colored red for ease of analysis. Several images of the UCNPs were merged with the single GFP image because the laser exposure spot size was small compared to the size of the organ. These results show the UCNPs selectively localize to the tumors instead of the healthy tissue.

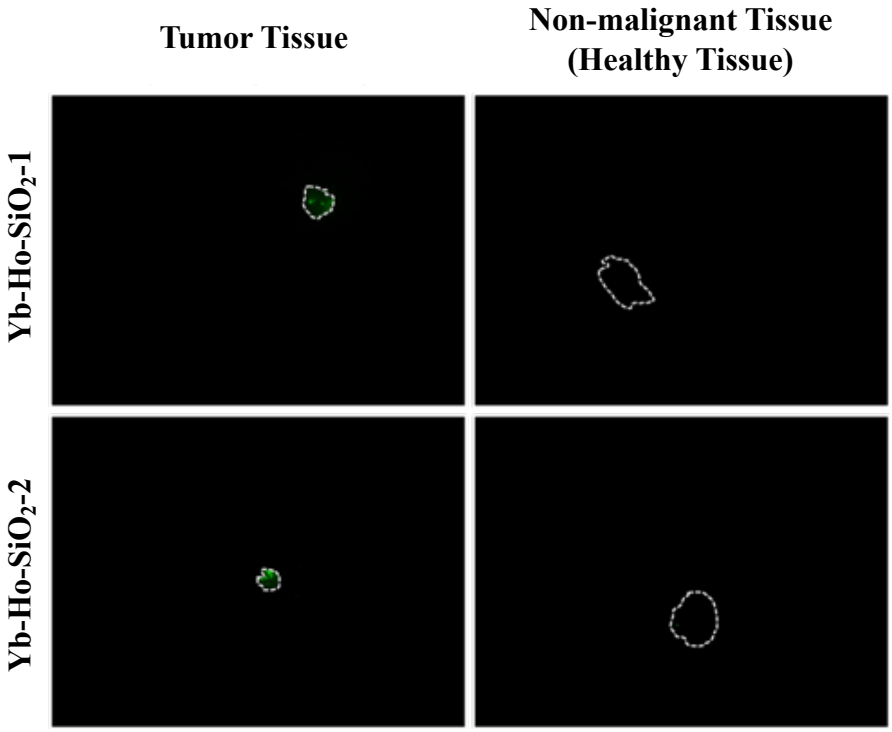


**Figure S7**: Fresh tumors and non-malignant tissues were obtained from patients and incubated ex-vivo with **Yb-Ho-$SiO_2$-1 and Yb-Ho-$SiO_2$-2** UCNPs that emit green, 545 nm light, and imaged with Leica Z16 dissection macroscope after 4 days. UCNPs selectively localized and labeled the metastatic tumor samples with minimal labeling of matched healthy tissue samples. Tumor and matched non-malignant samples were collected from 2 patients. Images were taken at a 4 second exposure time.

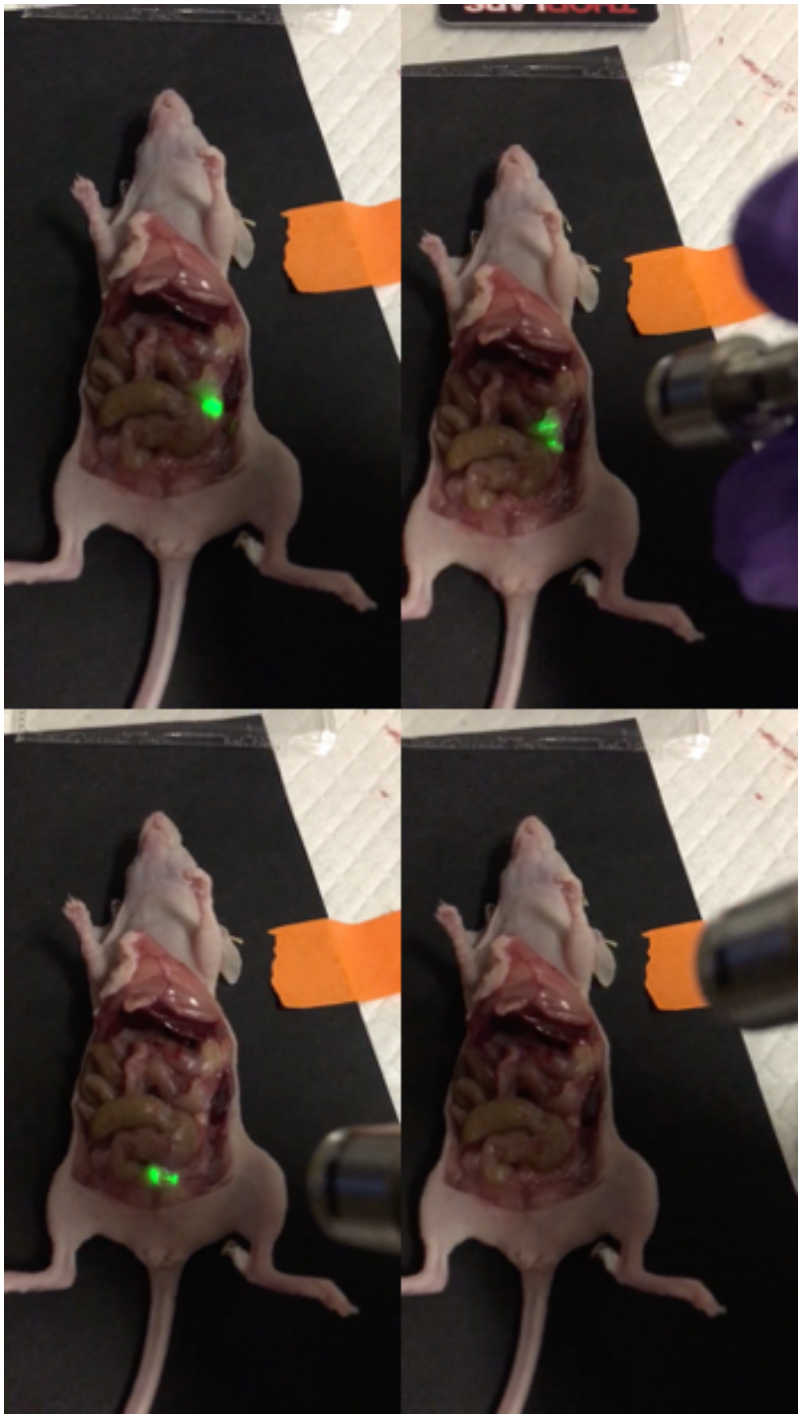

**Figure S8**: NIR guided imaging of ovarian cancer in a mouse. A typical protocol of injection and incubation with **Yb-Ho-$SiO_2$-2** produced visually bright emission of the cancer cells when exposed to 980 nm light. The bottom right figure shows that the healthy tissue does not fluoresce indicating they lack UCNPs as a result of tumor-specific uptake. A fiber optic coupled to a diffusor allowed for simple and rapid scanning of the abdominal cavity for tumor cells.

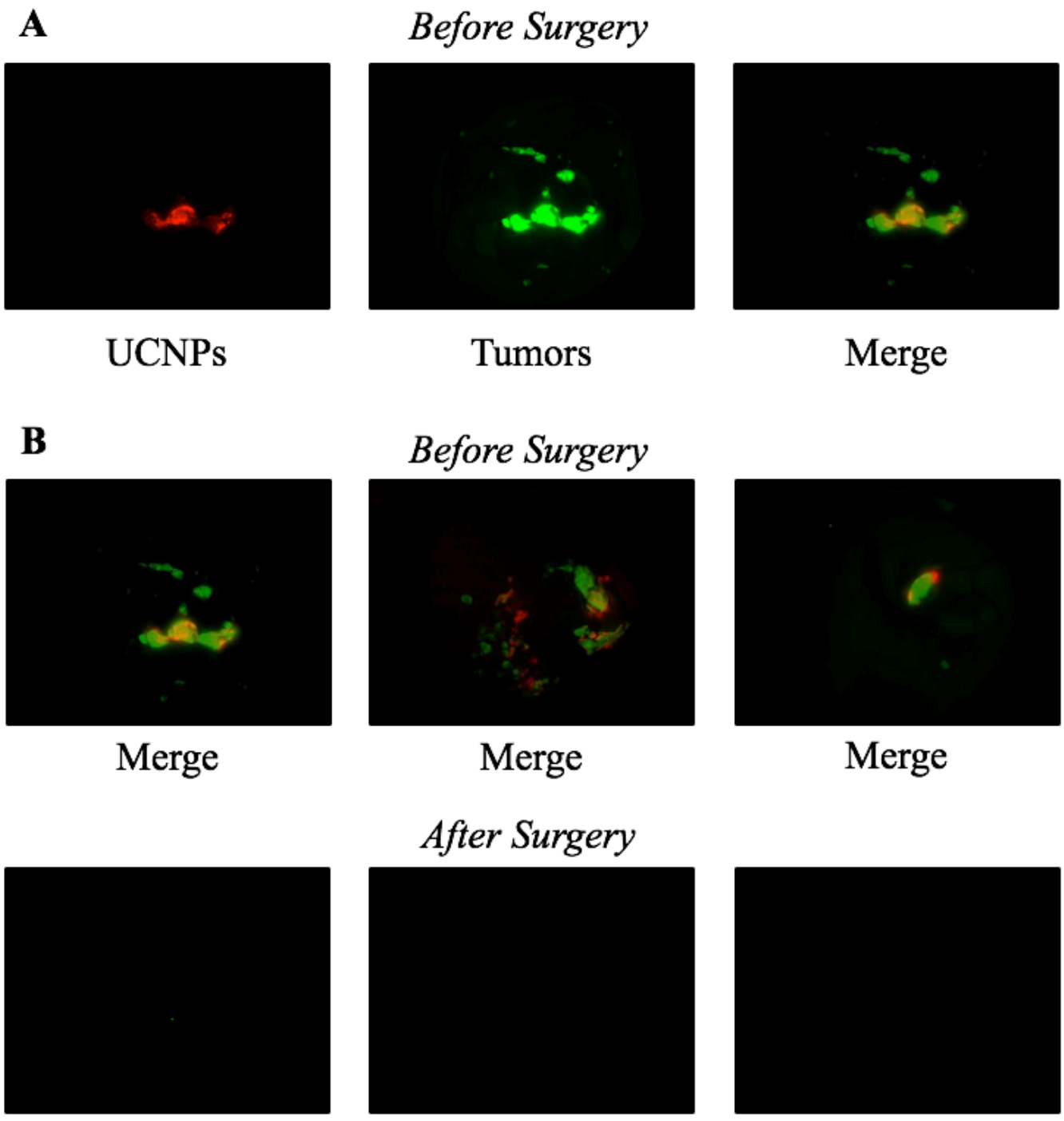


**Figure S9**: A) Example of imaging overlay before surgery of **Yb-Ho-SiO$_2$-2** emission (false colored red) and eGFP (green) from tumors. UCNPs are false colored red for clarity. Complete coverage of the tumor image with the UCNP is not achieved because the excitation areas are not equivalent. The UCNP excitation area is smaller than the confocal microscope. This is an engineering issue and can be fixed with a more optimized 980 nm laser setup. B) Several examples of before and after images of cancerous tissue

using surgery-guided techniques. In each example, nearly all the tumors are removed using the guided technique.

***Additional Discussions/Conclusions***

*Alternative Excitation Source – Moving from 980 nm to 808 nm*

We sought to use the "Yb,Ho@Yb@Nd@Y" construction as a way to shift our excitation source from 980 nm to 808 nm.[1–3] The UCNPs excitation wavelength of 980 nm comes from the large, narrow absorbance band of ytterbium (Yb) ions in the particle. Yb interacts well with emitters – holmium (Ho), the green emission here – making it the desired excitation source for UCNPs. Unfortunately, water absorbance at this wavelength (980 nm) is at a local maximum.[13–15] Recent studies have shown possibilities of shifting the UCNP excitation wavelength to 808 nm through the use of neodymium (Nd).[3,6,10,16,17] Neodymium (Nd) is responsible for the 810 nm absorption and "passes" its energy to ytterbium (Yb), which in turns "passes" it to the holmium (Ho) for emission. An 808 nm excitation region is a common target for biological applications since water is at a local minimum in this region. This shift in excitation wavelength would allow for greater laser powers to be used safely, which would be beneficial to both the emission intensity of the UCNPs and the safety from exposure.

UCNPs with specific layering constructions are synthesized using the above-mentioned procedure. A general design of Yb,Ho@Yb@Nd@Y was studied to increase green emission from an 808 nm excitation source. Separating the Nd from the Ho is important to prevent undesired decay pathways from the excited state. Several variants from this general design were also tested (**data not shown**): co-doping inert elements (Y or Ca) through the layers to generate crystal uniformity, varying the ratios of Nd and Yb present in the particle, and changing the composition of the outer protective shell (Y or Ca). After several iterations and combinations, large particles were made that had strong green emission with both 980 nm and 808 nm light sources. Unfortunately, after silica coating, the 808 nm excited particle lost much of their luminescence and were not able to produce a bright green emission (even with higher laser powers). The 980 nm excitation/emission still worked very well.

## References


(1) Zhong, Y.; Tian, G.; Gu, Z.; Yang, Y.; Gu, L.; Zhao, Y.; Ma, Y.; Yao, J. Elimination of Photon Quenching by a Transition Layer to Fabricate a Quenching-Shield Sandwich Structure for 800 Nm Excited Upconversion Luminescence of $Nd^{3+}$ -Sensitized Nanoparticles. *Advanced Materials* **2014**, *26* (18), 2831–2837. https://doi.org/10.1002/adma.201304903.

(2) Chen, G.; Damasco, J.; Qiu, H.; Shao, W.; Ohulchanskyy, T. Y.; Valiev, R. R.; Wu, X.; Han, G.; Wang, Y.; Yang, C.; Ågren, H.; Prasad, P. N. Energy-Cascaded Upconversion in an Organic Dye-Sensitized Core/Shell Fluoride Nanocrystal. *Nano Letters* **2015**, *15* (11), 7400–7407. https://doi.org/10.1021/acs.nanolett.5b02830.

(3) Wang, D.; Xue, B.; Kong, X.; Tu, L.; Liu, X.; Zhang, Y.; Chang, Y.; Luo, Y.; Zhao, H.; Zhang, H. 808 Nm Driven $Nd^{3+}$ -Sensitized Upconversion Nanostructures for Photodynamic Therapy and Simultaneous Fluorescence Imaging. *Nanoscale* **2015**, *7* (1), 190–197. https://doi.org/10.1039/C4NR04953E.

(4) Prorok, K.; Pawlyta, M.; Stręk, W.; Bednarkiewicz, A. Energy Migration Up-Conversion of $Tb^{3+}$ in $Yb^{3+}$ and $Nd^{3+}$ Codoped Active-Core/Active-Shell Colloidal Nanoparticles. *Chemistry of Materials* **2016**, *28* (7), 2295–2300. https://doi.org/10.1021/acs.chemmater.6b00353.

(5) Song, X.; Zhang, J.; Yue, Z.; Wang, Z.; Liu, Z.; Zhang, S. Dual-Activator Codoped Upconversion Nanoprobe with Core–Multishell Structure for *in Vitro* and *in Vivo* Detection of Hydroxyl Radical. *Analytical Chemistry* **2017**, *89* (20), 11021–11026. https://doi.org/10.1021/acs.analchem.7b02995.

(6) Zhang, Y.; Yu, Z.; Li, J.; Ao, Y.; Xue, J.; Zeng, Z.; Yang, X.; Tan, T. T. Y. Ultrasmall-Superbright Neodymium-Upconversion Nanoparticles via Energy Migration Manipulation and Lattice Modification: 808 Nm-Activated Drug Release. *ACS Nano* **2017**, *11* (3), 2846–2857. https://doi.org/10.1021/acsnano.6b07958.

(7) Johnson, N. J. J.; He, S.; Diao, S.; Chan, E. M.; Dai, H.; Almutairi, A. Direct Evidence for Coupled Surface and Concentration Quenching Dynamics in Lanthanide-Doped Nanocrystals. *Journal of the American Chemical Society* **2017**, *139* (8), 3275–3282. https://doi.org/10.1021/jacs.7b00223.

(8) Li, X.; Shen, D.; Yang, J.; Yao, C.; Che, R.; Zhang, F.; Zhao, D. Successive Layer-by-Layer Strategy for Multi-Shell Epitaxial Growth: Shell Thickness and Doping Position Dependence in Upconverting Optical Properties. *Chemistry of Materials* **2013**, *25* (1), 106–112. https://doi.org/10.1021/cm3033498.

(9) Li, Z.; Lv, S.; Wang, Y.; Chen, S.; Liu, Z. Construction of LRET-Based Nanoprobe Using Upconversion Nanoparticles with Confined Emitters and Bared Surface as Luminophore. *Journal of the American Chemical Society* **2015**, *137* (9), 3421–3427. https://doi.org/10.1021/jacs.5b01504.

(10) Shen, J.; Chen, G.; Vu, A.-M.; Fan, W.; Bilsel, O. S.; Chang, C.-C.; Han, G. Engineering the Upconversion Nanoparticle Excitation Wavelength: Cascade Sensitization of Tri-Doped Upconversion Colloidal Nanoparticles at 800 Nm. *Advanced Optical Materials* **2013**, *1* (9), 644–650. https://doi.org/10.1002/adom.201300160.

(11) Guo, Y.; Rogelj, S.; Zhang, P. Rose Bengal-Decorated Silica Nanoparticles as Photosensitizers for Inactivation of Gram-Positive Bacteria. *Nanotechnology* **2010**, *21* (6), 065102. https://doi.org/10.1088/0957-4484/21/6/065102.

(12) Chen, F.; Zhang, S.; Bu, W.; Chen, Y.; Xiao, Q.; Liu, J.; Xing, H.; Zhou, L.; Peng, W.; Shi, J. A Uniform Sub-50 Nm-Sized Magnetic/Upconversion Fluorescent Bimodal Imaging Agent Capable of Generating Singlet Oxygen by Using a 980 Nm Laser. *Chemistry - A European Journal* **2012**, *18* (23), 7082–7090. https://doi.org/10.1002/chem.201103611.

(13) Smith, A. M.; Mancini, M. C.; Nie, S. Second Window for in Vivo Imaging. *Nature Nanotechnology* **2009**, *4* (11), 710–711. https://doi.org/10.1038/nnano.2009.326.

(14) Macdonald, I. J.; Dougherty, T. J. Basic Principles of Photodynamic Therapy. *Journal of Porphyrins and Phthalocyanines* **2001**, *05* (02), 105–129. https://doi.org/10.1002/jpp.328.

(15) Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging. *Chemical Reviews* **2010**, *110* (5), 2620–2640. https://doi.org/10.1021/cr900263j.

(16) Sedlmeier, A.; Gorris, H. H. Surface Modification and Characterization of Photon-Upconverting Nanoparticles for Bioanalytical Applications. *Chemical Society Reviews* **2015**, *44* (6), 1526–1560. https://doi.org/10.1039/C4CS00186A.

(17) Park, Y. I.; Lee, K. T.; Suh, Y. D.; Hyeon, T. Upconverting Nanoparticles: A Versatile Platform for Wide-Field Two-Photon Microscopy and Multi-Modal in Vivo Imaging. *Chemical Society Reviews* **2015**, *44* (6), 1302–1317. https://doi.org/10.1039/C4CS00173G.