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Single upconversion nanoparticle imaging at sub-10 W cm⁻² irradiance

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Abstract

Lanthanide-doped upconversion nanoparticles (UCNPs) are promising single-molecule probes given their non-blinking, photobleach-resistant luminescence upon infrared excitation. However, the weak luminescence of sub-50 nm UCNPs limits their single-particle detection to above 10 kWcm⁻² that is impractical for live cell imaging. Here, we systematically characterize single-particle luminescence for UCNPs with various formulations over a 10⁶ variation in incident power, down to 8 Wcm⁻². A core-shell-shell (CSS) structure (NaYF₄@NaYb_{1-x}F₄:Er_x@NaYF₄) is shown to be significantly brighter than the commonly used NaY_{0.78}F₄:Yb_{0.2}Er_{0.02}. At 8 Wcm⁻², the 8% Er³⁺ CSS particles exhibit a 150-fold enhancement given their high sensitizer Yb³⁺ content and the presence of an inert shell to prevent energy migration to defects. Moreover, we reveal power-dependent luminescence enhancement from the inert shell, which explains the discrepancy in enhancement factors reported by ensemble and previous single-particle measurements. These brighter probes open the possibility of cellular and single-molecule tracking at low irradiance.

Optical upconversion uses two or more near-infrared photons in a multiple-step excitation process to generate emission at shorter wavelengths. This luminescence offers many applications including background-free imaging, optical sensing, multicolor displays and photovoltaics^{1–3}. Particularly, lanthanide ions such Er³⁺- and Tm³⁺-doped upconversion nanoparticles (UCNPs) have emerged as important luminescent nanoparticles for bio-

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Author contributions

Q.L., Y.Z., C.S.P., T. Y., and L.M.J. were responsible for the experimental work. Q.L., Y.Z., C.S.P. and S.C. conceived the project. S.C. supervised the research. All authors discussed the results. The manuscript was written by Q.L., Y.Z., C.S.P. and S.C.

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Data availability

The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

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imaging⁴ and photovoltaic applications⁵ due to their exceptional optical properties such as photostability, non-blinking, large anti-Stokes shifts and sharp emission lines^{3,5-9}.

While there are decades of research on UCNPs and numerous studies using UCNPs for bio-imaging², most optical studies have been made with large ensembles of particles. In order to better characterize the particles, optical measurements of isolated particles need to be correlated with electron microscope images that clearly resolve the particle size to confirm that the emission is from an individual particle and not a cluster of particles. Such measurements are also essential to be able to determine how the distribution in particle brightness correlates with the distribution of particle size and quality. For example, single-particle characterizations have revealed heterogeneity of UCNP crystal quality, as inferred by their spectral variations¹⁰. Despite the importance of single-particle imaging, very few studies have investigated the optical properties of single UCNPs¹⁰⁻¹⁶.

Even though UCNPs possess many desirable optical features, one of the major hurdles in the application of UCNPs as single-molecule biological probes is their weak luminescence. To date, reliable single-particle imaging of UCNPs have required high illumination power density in the range of 1 kW cm^{-2} to 10 MW cm^{-2} ¹⁰⁻¹⁵. However, prolonged illumination with 980 nm light at these intensities is known to induce photodamage in cells. Significant efforts have been put into improving the brightness by utilizing strategies such as optimizing the host matrix¹⁷⁻¹⁹ and increasing sensitizer^{20,21} or activator ions^{10,12}. Other efforts include adding organic dye to sensitize the upconversion process²², adding an inactive shell to reduce non-radiative quenching through vibrational modes of the solvent^{13,23-26}, and incorporating plasmonic resonance enhancement^{27,28}. Nevertheless, most of these experiments were ensemble measurements that may not necessarily lead to the optimal enhancement at the single-particle level. Furthermore, there is a wide range of reported enhancement factors even for the same design strategy. For instance, the luminescence improvement reported from the addition of an inert shell ranges from modest single-digit increase in brightness to over 100x enhancement^{13,23-26}.

In order to systemically optimize the upconversion luminescent brightness of UCNPs, we have employed a combination of wide-field and confocal microscopy to characterize a set of UCNPs at the single-particle level with laser power density between 8 W cm^{-2} to 6 MW cm^{-2} . We investigated the effect of particle size, the addition of an inactive shell, and variations in the concentration of activator. More importantly, to explore the possibility of luminescence enhancement with higher Yb^{3+} content, we have synthesized and measured the properties of a new core-shell-shell structure ($\text{NaYF}_4@ \text{NaYb}_{1-x}\text{F}_4:\text{Er}_x@ \text{NaYF}_4$).

We used the solvent thermal method²⁹ to synthesize a series of UCNPs with modifications to the most widely used Er^{3+} -emissive UCNPs (β -phase $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$, Supplementary Fig. S1). The transmission electron microscopy (TEM) images showed a uniform and monodispersed morphology (Supplementary Fig. S2a), with the size of $22.0 \pm 0.7 \text{ nm}$ (Supplementary Fig. S3a).

The low lying optical cross-section of Yb^{3+} is concentrated into a single $^2\text{F}_{7/2} \rightarrow ^2\text{F}_{5/2}$ transition, and rapid energy transfer among Yb^{3+} ions is used to step-wise excite Er^{3+} ions.

An obvious choice to create the most optically active UCNPs would be to synthesize a nanocrystal with an admixture of Yb^{3+} and Er^{3+} ions that contains no optically inactive Y ions. However, controllable synthesis of small (<30 nm) $\beta\text{-NaYbF}_4$ by one-step reaction remains a challenging task^{13,29}. In order to create size-tunable, Yb-rich nanoparticles with high crystal quality, we first synthesized 6.0 ± 0.6 nm diameter $\beta\text{-NaYF}_4$ seed crystals (Fig. 1a, 1d, Supplementary Fig. S4) that allow epitaxial growth of size-tunable $\beta\text{-NaYbF}_4$ ¹³. Having the same optically-active volume is required for a fair comparison of brightness between nanoparticles with different formulations. Therefore, a series of $\text{NaYF}_4@\text{NaYb}_{1-x}\text{F}_4:\text{Er}_x$ ($x=0.02, 0.04, 0.08, 0.16$, and 0.5) core-shell nanoparticles with all particles of uniform size ~ 22 nm were synthesized (Fig. 1b, 1d, Supplementary Fig. S5), which can be compared to the 22 nm core-only $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$. Note that the 6 nm seed crystal represents only 2% loss of the active luminescence volume.

Finally, it is well documented that high concentrations of sensitizer or activators can lead to concentration-quenching and hence decreased luminescence. Non-radiative energy loss can be alleviated by growing an epitaxial shell that reduces the resonant energy transfer among Yb^{3+} ions to the surface defects and to vibrational modes of the surrounding solvent^{13,23–26}. For this reason, we added a final inert NaYF_4 shell (~ 3 nm thickness). The resulting core-shell-shell β -phase $\text{NaYF}_4@\text{NaYb}_{1-x}\text{F}_4:\text{Er}_x@\text{NaYF}_4$ UCNPs has an overall size of around 28 nm (Fig. 1c-e, Supplementary Fig. S4, S5, S6, and S7). Scanning Transmission Electron Microscopy (STEM) and energy dispersive x-ray (EDX) mapping confirmed the presence of the Yb-rich core and the NaYF_4 shell (Fig. 1f and Supplementary Fig. S8).

Surface quenching significantly reduces the brightness of UCNPs, especially for smaller particles whose emission intensity no longer follows ideal volumetric scaling¹⁰. Consequently, we characterized two additional core-only $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ UCNPs with varying sizes: one close in size with the core-shell-shell structure (27.1 ± 0.8 nm, Supplementary Fig. S2b and S3b) and another larger UCNPs (33.6 ± 0.7 nm, Supplementary Fig. S2c and S3c). In order to suppress surface quenching, we also synthesized the canonical core-shell $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}@\text{NaYF}_4$ by growing an inert NaYF_4 shell on the 22 nm core-only UCNPs, giving an overall size of 29.1 ± 1.0 nm (Supplementary Fig. S2d and S3d). To examine the effect of the activator concentration, a set of ~ 22 nm core-only UCNPs were made with increasing Er^{3+} doping level from 2% to 16% while keeping the Yb^{3+} concentration at 20% (Supplementary Fig. S1, S2a, Fig. S9 and Fig. S10).

Figure 2a shows a representative ensemble upconversion luminescence (UCL) spectrum with 980 nm laser excitation of a solution containing 22 nm $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ UCNPs². Emission peaks centered at 520 nm, 541 nm, and 654 nm (Fig. 2a, 2b and Supplementary Fig. S11) are attributed to the transitions from the $^2\text{H}_{11/2}$, $^4\text{S}_{3/2}$, and $^4\text{F}_{9/2}$ excited states to the $^4\text{I}_{15/2}$ ground state, respectively. After growing a ~ 3 nm inert shell on these UCNPs, the UCL increased by 6.3 times while the spectrum remained unchanged (Fig. 2a). On the other hand, when an inert shell was added to $\text{NaYF}_4@\text{NaYb}_{0.98}\text{F}_4:\text{Er}_{0.02}$ to form the core-shell-shell structure, we observed an extraordinary 740-fold green emission enhancement (Fig. 2b). However, the seemingly more effective shell for NaYbF_4 could be due to more significant energy migration to the surface quenching sites with high Yb^{3+} content.

We examined the luminescence lifetimes as they are usually correlated with the particle brightness (Fig. 2c and Supplementary Table S1). Interestingly, the lifetime of $\text{NaYF}_4@\text{NaYb}_{0.98}\text{F}_4:\text{Er}_{0.02}$ (22.6 nm) is shorter than that of $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ (22.0 nm), suggesting that high level Yb^{3+} induced concentration quenching. However, similar lifetimes were observed when both types of particles were coated with an inert shell, implying that quenching at high level Yb^{3+} is predominantly due to energy migration to the surface defects³⁰ rather than self-quenching among Yb^{3+} ions. Concentration quenching was also found with increasing Er^{3+} content, which was exhibited as a reduced lifetime (Fig. 2c and Supplementary Table S1). This result presents a trade-off between concentration quenching and luminescence enhancement with higher activator content. The optimal Er^{3+} concentration for maximizing the brightness per unit volume in $\text{NaYF}_4@\text{NaYb}_{1-x}\text{F}_4:\text{Er}_x@\text{NaYF}_4$ UCNPs requires a comparison at the single-particle level because a precise determination of the UCNPs concentration in solution is nontrivial and could be easily biased by particle aggregation and precipitation. Also, a dense aggregation of particles could lead to partial shielding of surface quenching mechanisms.

To characterize the optical properties of single nanoparticles, the UCNPs in cyclohexane (400 ng/ml) were drop-cast onto a gridded coverslip with alphanumerically labeled photoetched squares for correlative luminescence and SEM (Fig. 3a, Supplementary Fig. S12 and S13). After correcting the wide-field fluorescence images by the laser illumination profile (Supplementary Fig. S13), the diffraction-limited fluorescent spots were carefully registered to corresponding positions on electron micrographs and each spot was examined at 200,000x magnification that is sufficient to clearly resolve and verify the size of the individual nanoparticles. We first compared the 8% Er-doped core-shell-shell UCNPs and the canonical 22.0 nm core-only $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ (Fig. 3a), which have the same optically active volume of ~22 nm. At a moderate power density of 625 W cm^{-2} (a typical wide-field microscope uses $1\text{--}10 \text{ kW cm}^{-2}$), the 8% Er CSS UCNPs emits ~20,000 photons per second (pps, obtained from the integrated volume of the 2D Gaussian fit), which is 5 times brighter than the standard core-only UCNPs (Fig. 3b).

When the power density was further lowered to 8 W cm^{-2} , the core-shell-shell UCNPs are drastically brighter than the standard core-only UCNPs by a factor of 150 (Fig. 3a and 3b), demonstrating single-particle imaging of sub-30 nm UCNPs at a power that is about five orders of magnitude smaller than what was previously used^{10–13}. Reliable single-particle detection requires a minimum signal to noise ratio (SNR) of $\sim 5^{30,31}$ (see Supplementary Notes). To achieve SNR of 5 at 8 W cm^{-2} , at least 15-sec long exposure was needed for the standard core-only UCNPs, while it took only 100 milliseconds for the core-shell-shell UCNPs. It is worth noting that most live whole animal imaging is performed with $\sim 100 \text{ mW cm}^{-2}$ ³². At these lower intensities, the core-shell-shell UCNPs are estimated to be ~5000-fold brighter than the core-only UCNPs (Supplementary Fig. S14). Although single particle tracking may be challenging *in vivo*, the overall detectability and ability to track the motion of a cell will be significantly improved with reduce nanoparticle dosage (10s to 100s of nanoparticles).

To further investigate the heterogeneity of single-particle luminescence, measurements on hundreds of individual nanoparticles were used to assemble brightness histograms (Fig. 3g,

Supplementary Fig. S15). For each type of UCNPs, about 20 field of views (FOVs) were rapidly recorded using wide-field illumination and an EMCCD camera. Figure 3c-f display representative luminescence images measured at 625 W cm^{-2} for 4 different UCNPs: 22.0 nm (Fig. 3c) and 27.1 nm (Fig. 3d) core-only $\text{NaY}_{0.78}\text{F}_4\text{:Yb}_{0.2}\text{Er}_{0.02}$, 29.1 nm core-shell $\text{NaY}_{0.78}\text{F}_4\text{:Yb}_{0.2}\text{Er}_{0.02}@\text{NaYF}_4$ (Fig. 3e), and 29.4 nm core-shell-shell $\text{NaYF}_4@\text{NaYb}_{0.92}\text{F}_4\text{:Er}_{0.08}@\text{NaYF}_4$ (Fig. 3f). Each histogram contains a main Gaussian peak, representing the UCL distribution of the single particles. Most of the diffraction-limited fluorescent spots were confirmed by the luminescence/SEM co-localization data to be individual UCNPs, with occasional appearance of dimers or small oligomers (Supplementary Fig. S16). The histograms from Supplementary Fig. S13 show that the fractional number of oligomers (the number of particles with brightness above the monomer brightness distribution) vary between 0.3% and 13%. The core-shell-shell structure is the brightest among the four types of UCNPs, but also with wider distribution. If the measured brightness distribution is solely due to the nanoparticle volume distribution, we would only expect a $\sim 12\%$ distribution. Considering different sources of variation add in quadrature, the measured $\sim 21\%$ luminescence distribution indicates a $\sim 17\%$ heterogeneity due to factors other than nanoparticle size (Supplementary Fig. S6), which could include doping inhomogeneity and crystal defects in the more complicated three-step core-shell-shell synthesis.

In order to systematically compare the brightness of the core-shell-shell structure to other UCNPs designed to enhance UCL, we measured the power-dependent luminescence curves (aka “saturation curves”) for single particles across nearly 6 orders of magnitudes in power density: $\sim 8 \text{ W cm}^{-2}$ to $\sim 6 \text{ MW cm}^{-2}$ (Fig. 4). Each saturation curve was obtained by averaging over at least 30 single particles within an FOV. The 8% Er-doped CSS UCNPs were found to be the brightest among all 12 types of UCNPs studied in this work across all power densities, and most importantly even brighter than the larger 33.6 nm core-only $\text{NaY}_{0.78}\text{F}_4\text{:Yb}_{0.2}\text{Er}_{0.02}$, by a factor of 21 at 8 W cm^{-2} . Fig. 4a and 4c compare the saturation curves for the core-only $\text{NaY}_{0.78}\text{F}_4\text{:Yb}_{0.2}\text{Er}_{0.02}$ with three different sizes (22.0 nm, 27.1 nm, and 33.6 nm), as well as the core-shell structure synthesized based on the 22.0 nm core. The emission intensities of the three core-only UCNPs scale linearly with particle volume (Fig. 4a and Supplementary Fig. S17, S18), indicating that good crystal quality was maintained for these particles as the larger particles were epitaxially grown from the smaller particles.

The core-shell UCNPs are brighter than its precursor 22 nm core-only nanoparticles (Fig. 4a), especially at intensities $< 1 \text{ kW cm}^{-2}$ (50-fold enhancement at 8 W cm^{-2}). However, the enhancement dropped to only ~ 1.5 above 10 kW cm^{-2} , and based on *volume-normalized* emission, the 22 nm core-only UCNPs are actually brighter than the 29 nm core-shell UCNPs at intensities $> 2 \text{ kW cm}^{-2}$ (Supplementary Fig. S18). This illustrates that the inert shell is effective in preventing luminescence quenching at low power, but the extra shell thickness becomes an overhead at higher irradiance where quenching of the core-only UCNPs is not as severe. This power-dependent luminescence enhancement of the core-shell structure could explain the one or two orders of magnitude higher enhancement factor obtained from the ensemble measurement compared to previous single-particle experiments, as ensemble measurement typically uses tens of W cm^{-2} while single-particle characterization with confocal microscopy employs $> 10 \text{ kW cm}^{-2}$. More importantly, the

inert shell in the core-shell-shell structure has greater luminescence enhancement—several hundred-fold at low power density and 4.4-fold at high power density (Supplementary Fig. S19). At the lower power density ranging from $\sim 8 \text{ W cm}^{-2}$ to $\sim 75 \text{ W cm}^{-2}$, the luminescence of the core-only UCNP exhibits a typical quadrature power dependence ($I \propto P^2$), consistent with a two-photon upconversion process (Fig. 4a and 4b). In contrast, when an inert shell is incorporated in the core-shell and core-shell-shell structure, a shallower slope of ~ 1.2 was observed for their saturation curves. This behavior suggests that UCNP with an inert shell are in an effectively higher power limit by preserving a high level of Yb^{3+} excited state population that would otherwise be mostly lost due to rapid energy transfer to surface quenching sites.

Fig. 4b and 4e illustrate the effect of Er^{3+} concentration on upconversion emission. As the Er^{3+} content increases from 2% to 16% in the core-only $\text{NaY}_{1-0.2-x}\text{F}_4:\text{Yb}_{0.2}\text{Er}_x$, the luminescence drops significantly at low power densities (Fig. 4b). In contrast, the trend reverses at $> 200 \text{ kW cm}^{-2}$ (Fig. 4e). This concentration quenching has been observed in both ensemble and single-particle measurements and agrees well with simulations reported by Gargas et al¹⁰. However, such concentration quenching is not so apparent in the core-shell-shell structure under low irradiance (Fig. 4c) where the luminescence of the 2% - 16% Er^{3+} -doped core-shell-shell UCNP is within a factor of 2 at 8 W cm^{-2} . This result again highlights the effectiveness of the inert shell in decreasing concentration-dependent quenching. At significantly higher Er^{3+} doping of 50% in the core-shell-shell structure, the upconversion emission dropped by a factor of 3.5 compared to the 8% doped UCNP. At higher power densities above 100 kW cm^{-2} , the brightness of the five core-shell-shell UCNP start to diverge (Fig. 4f), with a trend that is similar to the core-only UCNP with varying Er^{3+} doping. Nevertheless, the 8% Er^{3+} -doped core-shell-shell remains the brightest formulation tested in this power density region.

To put these single-particle measurements into perspective of other fluorescent probes, we have measured the upconversion quantum yield (UCQY) under 980 nm laser excitation at 120 W cm^{-2} (Supplementary Table S2). Consistent with the literature³³, the UCQY for our core-only UCNP is low and simply increasing the particles size did not provide much improvement, with $0.10 \pm 0.04\%$ and $0.15 \pm 0.05\%$ for the 22 nm and 34 nm UCNP, respectively. In contrast, UCQY was greatly increased to $5.03 \pm 0.60\%$ for the 29 nm core-shell structure due to reduced surface quenching²⁶. The brightest single-particle probe in this work, the core-shell-shell $\text{NaYF}_4@\text{NaYb}_{0.92}\text{F}_4:\text{Er}_{0.08}@\text{NaYF}_4$, was found to have a similar UCQY ($5.42 \pm 0.45\%$). However, the proper figure of merit in single-particle imaging is the emission rates at given illumination intensity because UCQY is calculated as the number of photons emitted divided by the number of photons absorbed. Our 8% Er core-shell-shell UCNP give the highest brightness due to not only a high UCQY but also a significant increase in 980 nm absorption with 92% Yb^{3+} .

In conclusion, we have systematically investigated and benchmarked various strategies designed to optimize the brightness of single UCNP. We tested various formulations and sizes of Er^{3+} -doped UCNP across ~ 6 orders of magnitude in power density. The luminescence brightness per unit volume of these particles is significantly increased by a core-shell-shell structure: a 6 nm diameter NaYF_4 core, an epitaxial shell of NaYbF_4 with x

% Er^{3+} substitution of Yb^{3+} , followed by a 3 nm thick NaYF_4 shell. In the parameter space studied, $x=8\%$ gives the best results. At an intensity of 8 W cm^{-2} , a 150-fold enhancement over the commonly used formulation of $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ is observed. This development opens up the possibility of photostable single-molecule tracking at very low irradiance, and even greater comparative sensitivity for cell identification and tracking in live animals.

Additionally, this study revealed complex behavior of single-particle brightness as a function of illumination power density, and resolves some of the seemingly contradictory enhancement factors or quantum yields in the literature. Specifically, it is shown why low power ensemble measurements cannot be directly compared to high power single nanoparticle measurements. Furthermore, this work demonstrates the value of quantitative and systematic characterization of laser power-dependent UCNPs brightness with well-calibrated absolute power density measurements. To this end, we developed a methodology that allows such unambiguous measurements at the single-particle level that provides a reliable experimental platform for continuing efforts in optimizing UCNPs tailored for various applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structural characterizations of core-shell-shell NaYF₄@NaYb_{0.98}F₄:Er_{0.02}@NaYF₄ UCNP.

TEM images of (a) seed NaYF₄, (b) NaYF₄@NaYb_{0.98}F₄:Er_{0.02}, and (c) core-shell-shell NaYF₄@NaYb_{0.98}F₄:Er_{0.02}@NaYF₄. (d) Size distributions at different stages during core-shell-shell nanoparticle synthesis. (e) High-resolution TEM image and (f) EDX elemental mapping of core-shell-shell NaYF₄@NaYb_{0.98}F₄:Er_{0.02}@NaYF₄.

Figure 2. UCL spectra and lifetime.

(a) Ensemble UCL spectra of $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ and $\text{NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}@ \text{NaYF}_4$ using 980 nm excitation at 20 W cm^{-2} . (b) UCL spectra of $\text{NaYF}_4@ \text{NaYb}_{0.98}\text{F}_4:\text{Er}_{0.02}$ and $\text{NaYF}_4@ \text{NaYb}_{0.98}\text{F}_4:\text{Er}_{0.02}@ \text{NaYF}_4$. (c) UCL decay of $\text{NaYF}_4@ \text{NaYb}_{1-x}\text{F}_4:\text{Er}_x@ \text{NaYF}_4$ ($x = 0.02, 0.04, 0.08, 0.16$) at 541 nm.

Figure 3. Correlative SEM and wide-field fluorescence images of UCNPs.

(a) SEM images and 3D representation of the wide-field UCL images at 8 W cm^{-2} and 625 W cm^{-2} of $\text{NaYF}_4@\text{NaYb}_{0.92}\text{F}_4:\text{Er}_{0.08}@\text{NaYF}_4$ (top) and $22\text{ nm NaY}_{0.78}\text{F}_4:\text{Yb}_{0.2}\text{Er}_{0.02}$ (bottom). Scale bars: 500 nm, and 30 nm for the inset. Different z-axis limits showing the UCL intensity (photons per second per pixel) were set at each irradiance. (b) Comparison of integrated single-particle UCL emissions. (c-f) Wide-field images taken at 625 W cm^{-2} for core (2% Er, 22 nm), core (2% Er, 27 nm), core-shell (2% Er, 29 nm), and core-shell-shell (8% Er, 29 nm), respectively. The same color map was applied to all four images. Scale bar: 2 μm . (g) Normalized UCL histograms for the UCNPs shown in (c-f). The histograms were built with multiple FOVs to get reliable statistics over hundreds of single particles. Solid line represents the Gaussian fit.

Figure 4. Single-particle saturation curves.

(a-c) Saturation curves at power densities from 8 W cm⁻² to 27 kW cm⁻² obtained with wide-field microscopy. The kink in the saturation curves under low power densities is due to the addition of an OD2 ND filter. (d-f) Saturation curves at power densities from 20 kW cm⁻² to 6 MW cm⁻² obtained with confocal microscopy (see Supplementary Fig. S20 for confocal images). (a, d) compares UCL for different sizes of core-only UCNP (2% Er) and a 29 nm core-shell UCNP grown from the 22 nm core. (b, e) compares UCL for core-only UCNP (22 nm) with different Er³⁺ doping. (c, f) compares UCL for core-shell-shell UCNP with different Er³⁺ doping. Notice that log-scale is used for the y-axis in (a-c) while linear-scale is used in (d-f). Error bars represent standard deviation from the mean.