Optical investigation of gold shell enhanced 25nm diameter upconverted fluorescence emission

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Abstract
We enhance the efficiency of upconverting nanoparticles by investigating the plasmonic coupling of 25 nm diameter NaYF₄:Yb, Er nanoparticles with a gold-shell coating, and study the physical mechanism of enhancement by single-particle, time-resolved spectroscopy. A three-fold overall increase in emission intensity, and five-fold increase of green emission for these plasmonically enhanced particles have been achieved. Using a combination of structural and fluorescent imaging, we demonstrate that fluorescence enhancement is based on the photonic properties of single, isolated particles. Time-resolved spectroscopy shows that the increase in fluorescence is coincident with decreased rise time, which we attribute to an enhanced absorption of infrared light and energy transfer from Yb³⁺ to Er³⁺ atoms. Time-resolved spectroscopy also shows that fluorescence life-times are decreased to different extents for red and green emission. This indicates that the rate of photon emission is not suppressed, as would be expected for a metallic cavity, but rather enhanced because the metal shell acts as an optical antenna, with differing efficiency at different wavelengths.

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(Some figures may appear in colour only in the online journal)

Introduction

Upconverting nanoparticles (UCNPs) are excited in the near infrared (NIR) and emit fluorescence in the visible spectrum, offer superior photostability, and high signal-to-noise ratio due to zero tissue autofluorescence [1–3]. It is believed that rare earth doped upconversion materials show little to no toxicity, depending on their surface coating. Previous in vitro and in vivo studies show minimal to no decrease of cell viability and no apparent symptoms of morbidity in mice [4–7].

These properties make UCNPs ideal for imaging and monitoring of biological processes over long time durations. UCNPs have been used in point-of-care diagnostics [8] such as in lateral flow assays [9–13], array-in-well assays [14] and in microarray assays [12]. In contrast to UCNPs, traditional fluorescence microscopy utilizes excitation in the ultraviolet (UV) or blue spectral region, and detection of a longer-wavelength, Stokes-shifted [15] fluorescence [16]. The use of the short-wavelength excitation leads to a lower penetration depth of the excitation light [17], and gives rise to auto-fluorescence, photobleaching and photodamage to biological specimens [18]. In addition, since upconversion is a two-photon fluorescence process, it has the same ability as other two-photon fluorescence microscopies to resolve the three-dimensional structure of objects [1].
Upconversion in UCNPs is based on the electronic transitions of the inner 4f-shell levels of the rare earth ions (figure 1). Many efficient particles reported over the last decade utilize a system with two or more rare-earth elements, in which one absorbs the majority of the excitation energy that is subsequently transferred to the other species [19–21], in a process known as the energy transfer upconversion (ETU) effect. Transition from the higher excited state to the ground state then leads to fluorescence which is observed as anti-Stokes emission whereby the emitted photon is higher in energy than the excitation photons individually [15]. UCNPs operate via real, long-lived intermediate states, which result in excited state lifetimes of tens to hundreds of microseconds [22].

Despite the fundamental advantages that UCNP have over semiconductor nanoparticles and molecular dyes, they have not been used widely due to their comparatively low brightness and upconversion efficiency at low pump powers. The reason lies in the low absorption cross-section for infrared light, which is caused by transitions that are only weakly allowed, the same physical effect gives rise to the long lifetimes that enable the upconversion process. However, the efficiency of UCNPs strongly increases with the intensity of the excitation laser, and so it has been suggested to locally increase the exciting electric fields. Recently, there has been interest in enhancing Anti-Stokes emission by coupling gold nanoparticles and gold shells to rare earth ion doped upconverting cores [21, 23–27], resulting in enhanced overall emission intensity, modification of the relative green over red emission [21, 23, 25, 26] and the possibility of bimodal imaging [21]. The enhancement is attributed to the tuning of the surface plasmon resonances to coincide with the excitation of the rare earth doped core. All these studies have focused on ensemble optical measurements performed in solution.

Our investigation focuses on single UCNPs of a biologically relevant size of about 25 nm in diameter. We follow the approach of plasmonic enhancement by depositing a gold shell on UCNPs to directly increase the absorption efficiency. Our earlier work focuses on gold shell coupling to large UCNPs with a 350 nm diameter doped core and a 60 nm thick undoped NaYF4 shell [28], in order to eliminate strong coupling effects, such as quenching of fluorescence from rare earth ions through destructive interference on the gold shell. Since there was no strong coupling of the rare earth ions to the gold shell, time-resolved fluorescence studies were not explored in the previous work. In this previous work, electric field enhancement leading to excitation enhancement only was observed. In this report, despite the small size of the investigated UCNP, through single particle time-resolved studies, we show that a gold shell coating leads to enhancement attributed to both excitation and emission enhancement processes. In the small UCNPs, the overall emission intensity is increased three-fold at the single particle level, and green emission is enhanced up to five times. A single particle study of this depth has not been demonstrated by other groups.

In our exploration of the mechanism of enhancement, we only use single particles. This distinction is critical, as the intended application is as well-dispersed single-particles that can penetrate tissues and cells, and imaging at the single cell level. Schietinger et al [23] has investigated the effect of gold nanoparticle coupling to upconverter cores at the single particle level, but not that of gold shell coupling to the upconverter core. Furthermore, the distinction between single particles and bulk powders is very important as aggregated gold shell UCNPs show no significant enhancement of the green emission intensity compared to single UCNPs (see supplementary data) and also a change in green/red emission ratio, as a result of the interference of electric fields between such particles [28]. Furthermore, prior studies [29, 30] have shown that bare UCNPs optimized for bright emission in ensemble measurements are in fact dim under the tightly focused excitation and high laser intensity used in single particle imaging. The high intensity leads to a competition between upconversion and linear decay processes, leading to observable saturation effects [31–33]. Moreover, the localized heating under high laser fluence contributes to thermal quenching of the emission [34, 35].

We have developed a rigorous single nanoparticle spectroscopy protocol. This enables us to perform correlated structural and time-resolved optical single nanoparticle spectroscopy under nanosecond pulsed-laser excitation. For a subset of our particles, we correlate the emission and time-resolved studies on single particles with AFM of the same particles. We find that the rise times for green and red emissions are shortened. This is consistent with the original concept of plasmonic local infrared intensity enhancement and the two-photon nature of the upconversion process. However, we are not able to attribute this change in rise time to the local intensity enhancement, or from a possible change
in the Yb\textsuperscript{3+} to Er\textsuperscript{3+} energy transfer rates. Surprisingly, we also find that the fluorescence lifetimes of the green emission is considerable shortened in presence of the gold layer, while the lifetime of red emission is essentially unchanged. Together with the stronger enhancement of green light, we thus conclude that the emission rate of green light is selectively enhanced by the presence of the gold shell. This is remarkable, as one would intuitively anticipate that a far-sub-wavelength metallic cavity would be non-resonant with both the excitation and emission wavelength. Our results show that the enhancement of fluorescence is due to a doubly resonant process where both excitation as well as emission, are enhanced through the presence of the metallic shell.

**Methods**

The NaYF\textsubscript{4}:Yb, Er nanoparticles were synthesized according to the procedure outlined in our prior work, with a molar concentration of 20% Yb\textsuperscript{3+} and 2% Er\textsuperscript{3+} [36]. After washing in ethanol, the UCNPs are coated with a silica shell [37], followed by silanization with amino propyltriethoxysilane to enable gold seed attachment [38]. Growth of the gold shell occurs through the growth and coalescence of individual gold seeds [27, 39–42]. To control gold shell thickness and to circumvent aggregation, we add 0.1 mM of thioglycolic acid in ethanol, the UCNPs are coated with a silica shell except for

\[
\frac{dN_{1,Yb}}{dt} = W_0N_{1,Yb}N_{0,Er} + W_{1,Yb}N_{1,Yb}N_{2,Er} + W_{2,Yb}N_{1,Yb}N_{1,Er} + R_{1,Yb}N_{1,Yb},
\]

\[
\frac{dN_{1,Er}}{dt} = W_0N_{1,Er}N_{0,Er} - W_{1,Er}N_{1,Er}N_{2,Er} - W_{2,Er}N_{1,Er}N_{1,Er} - R_{1,Er},
\]

\[
\frac{dN_{0,Er}}{dt} = -W_{0,Er}N_{0,Er} + R_{4,Er}N_{4,Er} + R_{5,Er}N_{5,Er} + R_{6,Er}N_{6,Er},
\]

\[
\frac{dN_{1,Er}}{dt} = -W_{0,Er}N_{1,Er}N_{1,Er} + \beta N_{2,Er},
\]

\[
\frac{dN_{2,Er}}{dt} = -W_{0,Er}N_{2,Er} - W_{1,Er}N_{1,Er}N_{2,Er} - \beta N_{2,Er} - W_{2,Er}N_{2,Er}N_{2,Er} - R_{2,Er}N_{2,Er},
\]

\[
\frac{dN_{3,Er}}{dt} = W_{2,Er}N_{1,Er}N_{1,Er} - R_{4,Er}N_{4,Er} + \beta N_{5,Er},
\]

\[
\frac{dN_{5,Er}}{dt} = \beta N_{6,Er} - N_{5,Er} + R_{5,Er}N_{6,Er} + \beta S_{5,Er},
\]

\[
\frac{dN_{6,Er}}{dt} = W_{1,Er}N_{1,Er},
\]

where, \(N_{1,Yb}\) and \(N_{1,Er}\) refers to the \(^2F_{7/2}\) and \(^2S_{5/2}\) levels of the Yb\textsuperscript{3+} ion. \(N_{0,Er}\), \(N_{1,Er}\), \(N_{2,Er}\), \(N_{3,Er}\), \(N_{4,Er}\), \(N_{5,Er}\), \(N_{6,Er}\) and \(N_{5,Er}\) refers to \(4_{15/2}, 4_{13/2}, 4_{11/2}, 4_{9/2}, 2_{7/2}\) (narrowly spaced, treated as one) and \(^2F_{7/2}\) levels of the Er\textsuperscript{3+} ion. \(W_0, W_1, W_2\) are the rates of energy transfer between Yb\textsuperscript{3+} and Er\textsuperscript{3+} ions; \(N_{1,Er}\) to \(N_{0,Er}\), and \(N_{0,Er}\) to \(N_{2,Er}\), \(N_{1,Er}\) to \(N_{0,Er}\) and \(N_{2,Er}\) to \(N_{0,Er}\); \(N_{1,Er}\) to \(N_{0,Er}\) and \(N_{2,Er}\) to \(N_{0,Er}\) respectively. \(W_0\) is the energy transfer rate for Er\textsuperscript{3+} to Er\textsuperscript{3+} cross-relaxation from \(N_{2,Er}\) to \(N_{6,Er}\) and \(N_{2,Er}\) to \(N_{0,Er}\); \(R_{1,Er}\) and \(R_{2,Er}\) refers to the radiative transitions from the \(N_{1,Er}\) to \(N_{0,Er}\) and \(N_{2,Er}\) to \(N_{0,Er}\) respectively; \(R_{4,Er}\) and \(R_{5,Er}\) refers to the radiative transitions from \(N_{4,Er}\) to \(N_{0,Er}\) and \(N_{5,Er}\) to \(N_{0,Er}\) respectively; \(\beta_2, \beta_3\) and \(\beta_4\) refer to the non-radiative relaxations from \(N_{2,Er}\) to \(N_{1,Er}\), \(N_{5,Er}\) to \(N_{4,Er}\) and \(N_{6,Er}\) to \(N_{4,Er}\) respectively.

We attempted to fit the fluorescence decay curves in this publication using the full set of transitions, but find that the following minimal set gives good reproduction of the decay curves. In particular, we fit to Er: \(^2H_{11/2}, ^2S_{3/2}\) to \(^4I_{15/2}\).
Results

Figure 2(a) shows a TEM image of the as-synthesized, unmodified UCNP of about 25.2 ± 4.4 nm in diameter. Figure 2(c) shows the initial high density, ultra-small gold seeds adhered on a 12 nm thick silica shell (figure 2(c)) with a UCNP/silica diameter of 50.0 ± 5.8 nm (figure 2(b)) that are needed to produce uniform shell thicknesses and particle to particle consistency. Figure 2(d) shows a finished gold shell coating of about 12 nm on a UCNP (UCNP/Au shell diameter of 73.8 ± 4.7 nm). In figure 2(d), a dark outline can be seen in the magnified view, indicating a semi-continuous gold shell.

The aggregation of the gold shell coated UCNPs were a result of the direct pipetting of the gold shell coated UCNPs in water, which were allowed to dry in air. The slow drying rate caused the nanoparticles to move towards the center of the grid and aggregate. In our optical studies, the nanoparticles were immobilized on a quartz glass substrate with shaking, hence minimizing aggregation.

The progress of the gold shell growth on the UCNPs was monitored using absorption spectroscopy (figure 3). The initial plasmon peak of the gold seeds is at 520 nm. We grew the gold shells for 20 min, and absorption measurements were performed right after each growth, with thioglycolic acid addition to the measured sample, but without buffer exchange to DI water.

We have performed a series of gold shell growth on the UCNPs with increasing gold salt concentrations. We find that at the critical gold salt concentration of 7.5 × 10^{-4} M, the main plasmon peak suddenly shifts from the peak characteristic for separated seeds (λ ∼ 545 nm) to one that is indicative of coalesced neighboring particles with a significantly red-shifted peak into the NIR. The dramatic red shift is associated with the completion of the gold shell. However, if the shell is allowed to grow thicker after completion, the resulting plasmon resonance may shift towards the blue end of the spectrum [39]. The observed data is that of an ensemble of gold-shell nanoparticles, under Brownian motion, with each nanoparticle randomly oriented in solution. The broadened absorption peak is attributed to the shape anisotropy of our nanoparticles, resulting in a strong surface plasmon resonance dependence on nanoparticle orientation.

We have developed a protocol for correlating the structural and optical properties of single nanoparticles. We have performed this study on bare UCNPs, as the wide field fluorescence images of gold shell coated UCNPs frequently present non-fluorescent nanoparticles due to damaged nanoparticles and gold shell fragments due to aggressive probe tip sonication. From our experience with the bare UCNPs, aggressive probe tip sonication was necessary to optimize single particle distributions, particularly at such small nanoparticle sizes.

Figure 4(a) shows the wide field upconverted fluorescence images taken of a sample where the bright spots (A1 and A2) that are of higher intensities are clearly visible as
aggregates. For our single particle studies these brighter clusters are not considered in our measurements.

The AFM image in figure 4(b) shows an overview scan of the same particles shown in the zoomed wide field fluorescence image in figure 4(a). To collect the fluorescence emission spectra and decay measurements from single UCNP, smaller AFM scans were performed to distinguish between UCNP aggregates (A1, A2) and single UCNP (P1) (insets in figure 4(b)).

The FWHM in the profile analysis of the individual UCNP (P1) in figure 5(a) is closely correlated to the diameter of the UCNP measured by TEM. The larger FWHM is attributed to the sides of the AFM probe tip, which causes apparent broadening of the scanned feature.

From the AFM image shown in figure 5, small residues can be observed which are attributed to the cyclohexane solvent (black arrow). The UCNP were dispersed in cyclohexane and immobilized on a quartz glass substrate with an alpha-numerically indexed grid which leaves behind small residues distinctively smaller than the UCNP. Similar residues are observed by AFM imaging, where only cyclohexane without UCNP were immobilized the same way on quartz glass substrates (see supplementary data). We have no indication that they emit any light in the green or red spectral range. Indeed, UCNP are dispersed so sparsely that more than 99% of the sample surface show no upconversion fluorescence at all despite the presence of residues over most of the substrate.

Figure 6 shows the time-resolved decay recorded for P1 at (a) \( \lambda = 545 \) nm, and (b) \( \lambda = 650 \) nm. The Er\(^{3+} \) green emission at \( \lambda = 545 \) nm is attributed to the \( ^4S_{3/2} \) to the ground state \( ^4I_{15/2} \) transition, while the red emission at \( \lambda = 650 \) nm is attributed to the \( ^4F_{9/2} \) to the ground state transition. The green decay has a shorter rise time and decay time compared to the red decay. We also observe the \( \lambda = 525 \) nm fluorescence decay (not shown) attributed to the \( ^2H_{11/2} \) to \( ^4I_{15/2} (\lambda = 525 \) nm) transition, but do not study it in detail since it is weaker in intensity than the \( \lambda = 545 \) nm fluorescence.

Identical methods of nanoparticle dispersal (at the same concentration) and immobilization were carried out for UCNP coated with a gold shell. We relied on our experience with the bare UCNP, where small dim fluorescent spots were statistically most probable to be single particles.

Figure 7 shows the single particle emission spectra comparing UCNP with and without a gold shell. We find that
A gold shell thickness of about 12 nm leads to an overall emission intensity enhancement of about three times. Our earlier calculations on larger sized UCNPs suggest an optimum gold shell thickness of 10 nm [28], at which a balance between radiation reaching the core and fluorescence emanating is achieved. The largest enhancement occurs for the green emission at $\lambda = 545$ nm, with a five-fold increase compared to the uncoated UCNP. An enhancement was also observed in red emission at $\lambda = 650$ nm, but to a lesser extent, of up to two times only. We observe the green (545 nm)/red (650 nm) emission ratios of the gold shell nanoparticles varying from sample to sample, from 1.1 to 1.5.

In contrast, silica coated UCNP show a green/red ratio of roughly 0.6, which is consistent over a large number of measured nanoparticles. We believe that the variability in the observed green/red ratios can be attributed to the orientation of individual non-spherical gold-shell nanoparticles with respect to that of the incoming NIR excitation [28, 44, 45] contributing to varying strengths of plasmonic enhancement. The variable green/red ratios are also attributed to shattered gold shells due to aggressive probe tip sonication. We emphasize that vigorous probe tip sonication is essential to resolving single particles, but the trade off is damage to some particles.

To understand the details of the plasmonic enhancement, we performed time-resolved decay measurements on the same particles as shown in figure 7 at (a) $\lambda = 545$ nm, and (b) $\lambda = 650$ nm (figure 8). Both the emission decay rate and the rise time of the green 545 nm line (figure 8(a)) are reduced in the presence of a gold shell from 182 $\mu$s to 143 $\mu$s and from 40 $\mu$s to 21 $\mu$s (time to maximum), respectively. In figure 8(b), we find that both rise and decay time of the 650 nm (red) emission are less affected by gold coupling in relation to the total decay time (decay time 313 $\mu$s–278 $\mu$s, rise time 113 $\mu$s–73 $\mu$s, respectively).

**Discussion**

These observations can be obtained both in the framework of path A regardless of whether path B (figure 1) towards red emission is active or not. The 545 nm line is attributed to radiative decay from the $^4S_{3/2}$ level. The level is populated by promotion from the intermediate $^4I_{11/2}$ level to the $^4F_{7/2}$ level through resonant transfer, labeled A in figure 1. The energy transfer rate describes the rate of photon absorption by an ytterbium ion and subsequent transfer of the photon energy to a nearby erbium ion, known as the accepted ETU mechanism [33]. Afterwards, the non-radiative relaxation process from the $^4F_{7/2}$ to the $^2H_{11/2}$ level, and further to the $^4S_{3/2}$ level, is very efficiently coupled through the emission of phonons, where subsequent 545 nm emission occurs [46, 47]. In measurements of rise time versus increasing pump power, we observed a dramatic shortening of rise time (supplementary data). Thus, the rise time is limited only by the $^4I_{11/2}$ to $^4F_{7/2}$ transition. We interpret the rise time reduction at $\lambda = 545$ nm either as an increase in the amount of photon absorption by Yb$^{3+}$ ions (equivalent to two-fold power increase), or a large increase in cross-section of the $^4I_{11/2}$ to the $^4F_{7/2}$ transition.
through coupling via the particle plasmon mode. Our model cannot distinguish between these two effects. The reduction in lifetime of the green emitting $^4S_{3/2}$ level is attributed to a significant increase of the coupling constant to the ground state. An increased coupling to the $^4F_{9/2}$ level appears unlikely due to the increased green emission. We note that the presence of the gold shell leads to dissipation of the outgoing radiation from the rare earth dopants, and our calculations show that approximately 15% of the radiative output is absorbed in the gold shell [28]. However, the enhancement of the excitation efficiency outweighs the attenuation.

Red emission at $\lambda = 650$ nm occurs from the $^4F_{9/2}$ level, which can be either populated through a cascade from $^4S_{3/2}$ (path A), or decay from $^4I_{11/2}$ to $^4I_{13/2}$, followed by transfer of another photon (path B). The red/green branching point in path A is at $^4S_{3/2}$. Once the $^4S_{3/2}$ state is excited, either a 545 nm photon may be emitted, or a subsequent non-radiative phonon assisted relaxation may occur to the $^4F_{9/2}$ state where a 650 nm emission will occur. The branching ratio at the $^4S_{3/2}$ state is not pump power sensitive, but the initial population of the $^4S_{3/2}$ state is indeed sensitive to pump power. Thus, the observed preferential enhancement of green would be the result of the enhanced green emission rate attributed to path A. The red/green branching point in path B is at the $^4I_{11/2}$ level. At high photon flux (see figure 1), the $^4I_{13/2}$ to $^4F_{9/2}$ transition becomes more rapid, and red rise times in path B are limited by the lifetime of $^4I_{13/2}$. The decay constant for $^4I_{13/2}$ is $\sim 1/75 \mu s$ for a bare particle and $\sim 1/45 \mu s$ with gold shell, but it will in general depopulate faster as upconversion populates $^4F_{7/2}$ due to the previously mentioned enhanced cross section or transfer efficiency. Typically, as observed by other researchers [30, 48], higher pump powers favor red over green emission. The corresponding higher pump power intensities associated with single particle spectroscopy used in our work, combined with the effective higher electric field inside the gold-shell particles plays a role in the rebalancing of the emission spectrum. In this regard, the enhanced decay rate of green emission in the core–shell particle results in the green dominant spectra shown above for gold shell particles.

Figure 8. Time-resolved decay at $\lambda = 545$ nm (a) and $\lambda = 650$ nm (b) of single UCNPs with a gold shell (bold line) and silica shell coating only (dashed line) with corresponding fit using the rate equation model (open triangles and squares).

Note that aggregation of gold shell coated particles would modify the electric field enhancement [28, 49]. However, rise and decay times were consistent between gold shell coated particles, which is an indication of the effectiveness of our sample dispersion and immobilization methods. Indeed, gold shell coated aggregates in our measurements show a green/red ratio smaller than 1.0 in our earlier work [28]. A small variation of brightness from particle to particle was observed, likely due to a shape anisotropy of the hexagonal core [28, 44]. We accounted for this by discarding very dim particles that are most likely not optimally oriented with respect to the incident excitation.

Conclusions

We have shown, specifically through single particle measurements, that large enhancements in upconverted emission can be observed, with up to five times enhancement in the green, in individual nanoparticles, with optimal incident excitation direction and nanoparticle orientation with respect to the incident excitation. Coupling of a gold shell at optimal shell thickness, gives rise to excitation resonant to the intermediate $^4I_{11/2}$ level of the Er$^{3+}$ ion in UV–vis spectra. The transition rates of both green and red emissive levels are enhanced, with green to a greater extent, as indicated by much larger enhancements in green compared to red emission. Time-resolved measurements support the assertion that the mechanism of the gold shell enhancement of upconverted emission is due to the plasmon resonant amplification of the coupling of the intermediate levels of the Yb$^{3+}$ and Er$^{3+}$ ions. This enhances the overall signal and the photon transition rates compared to the (unchanged) phonon transition rates. The modification of transition rates explains the relative enhancement of green and red levels.
The presented results reveal novel insights into the upconversion process as it is modified by a change in local electric field density. These single particle studies give important information for the application of gold shell coated UCNPs for single cell and molecule studies.

Acknowledgments

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