ABSTRACT: Lanthanide-doped upconversion nanoparticles (UCNPs) have rich photophysics exhibiting complex luminescence kinetics. In this work, we thoroughly investigated the luminescence response of UCNPs to excitation pulse durations. Analyzing this response opens new opportunities in optical encoding/decoding and the assignment of transitions to emission peaks and provides advantages in applications of UCNPs, e.g., for better optical sectioning and improved luminescence nanothermometry. Our work shows that monitoring the UCNP luminescence response to excitation pulse durations (while keeping the duty cycle constant) by recording the average luminescence intensity using a low-time resolution detector such as a spectrometer offers a powerful approach for significantly extending the utility of UCNPs.

Lanthanide-doped upconversion nanoparticles (UCNPs) can convert low-energy near-infrared (NIR) photons into higher-energy NIR, visible, or even ultraviolet photons through subsequent absorption and accumulation of the energy of two or more excitation photons. Lanthanide photon upconversion circumvents the need for high-intensity short-pulse lasers, required in conventional coherent nonlinear optical processes, and thereby provides unique advantages and opportunities for applications. With the advances in upconversion nanochemistry and nanophotonics during the past two decades, UCNPs have been developed as an important group of luminescent nanomaterials with many applications in fields such as nanomedicine, photonics, and information technology.

UCNPs are composed of a large number of optical centers (lanthanide dopants) accommodated in a host lattice. The mutual interactions between these optical centers following light excitation make each nanoparticle operate as a kinetic optical system, exhibiting fascinating responses to various optical stimuli in the spectral, polarization, intensity, and time domains. For instance, in the time domain, the luminescence of UCNPs typically exhibits complex kinetics, featuring rise and decay processes encoded with rich information upon multiphotonic excitation with a short pulse.

Pulsed excitation is a commonly used approach in the investigation and applications of luminescent materials. As a distinguished example, transient state (TRAST) spectroscopy/microscopy based on pulsed excitation makes it possible to monitor photoinduced, dark state kinetics of fluorescent molecules in a widely applicable manner, by analyzing how the plain, time-averaged fluorescence intensity from organic fluorophores, or even autofluorescent compounds, changes upon systematically varying the modulation of the excitation light. Using the excitation pulse duration response of fluorophores, the TRAST concept has proven to be a powerful tool in cellular and molecular studies, for monitoring sparse molecular interactions, local oxygenation, and redox conditions in cells and solutions, via the dark state transitions of the fluorophores. This information is often difficult to obtain by detecting regular fluorescence parameters, if possible by other methods, before the introduction of the TRAST concept.

Various approaches based on pulsed excitation have also proven to be useful for the characterization of UCNPs and for developing their applications. For example, due to the nonlinear excitation rate dependence, quasi-continuous-wave (millisecond pulse duration) excitation of UCNPs has proven to be superior in several applications, such as UCNP-based biomedical imaging and UCNP optogenetics. Pulsed excitation has also proven to be useful in applications tuning the emission color of UCNPs and for selectively switching the high-order multiphotonic emission of UCNPs on and off. However, despite these studies, the pulsed excitation response

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of UCNPs has not been systematically investigated and its applications have remained underexplored.

In this work, we adapted an excitation pulse duration response procedure from TRAST spectroscopy of organic fluorophores to thoroughly investigate the rich photophysics of Yb\(^{3+}\)-Er\(^{3+}\)-codoped and Yb\(^{3+}\)-Tm\(^{3+}\)-codoped UCNPs. By experiments and numerical simulations, important parameters that can affect the excitation pulse duration response of UCNPs were identified and their effects were analyzed. Our study shows that a systematic variation of the excitation pulse duration can be very informative and have important implications in the characterization and applications of UCNPs, such as in the assignment of transitions of different emission peaks, for bioimaging, and in luminescent nano-thermometry.

The response of UCNPs to pulsed excitation can be investigated and examined in many ways, varying several different parameters of the pulsed excitation (e.g., pulse duration, duty cycle, and frequency) and monitoring and/or quantifying different changes in the upconversion luminescence (UCL) signal. In this work, we, following the TRAST concept, applied on organic fluorophores, confined ourselves to study how the average UCL intensity, \(\langle \text{UCL}(\lambda, w) \rangle \), for different emission bands of UCNPs at wavelength \(\lambda\) varied with pulse width \(w\) while keeping the duty cycle \(\eta\) of the square-wave pulse constant (see Figure 1a). During the measurements, UCNPs were irradiated by square-wave modulated 980 nm laser light, and the UCL intensity was recorded by a spectrometer with a sufficiently long exposure time to properly average the UCL photons emitted over multiple periods. The OFF time in a pulse period was carefully controlled to allow UCNPs to completely relax to the ground state after the photoexcitation during the ON time, to eliminate pile-up effects, i.e., remaining excited state populations between adjacent excitation pulses. The recorded UCL intensity was normalized to approach unity at long pulse widths by

\[
\langle \text{UCL}(\lambda, w) \rangle_{\text{norm}} = \frac{\langle \text{UCL}(\lambda, w) \rangle}{\langle \text{UCL}(\lambda, w_{\text{long}}) \rangle}
\]

and the dependence of \(\langle \text{UCL}(\lambda, w) \rangle_{\text{norm}}\) on \(w\) was plotted to yield an excitation pulse duration response curve, also termed a TRAST curve in previous studies of organic fluorophores. \(^{11}\)

This approach was first applied to three Yb\(^{3+}\)-Er\(^{3+}\)-codoped \(\text{NaYF}_4\) nanoparticle samples, synthesized using previously reported protocols with minor modifications. \(^{19,20}\) These nanoparticles have different sizes and shapes, as characterized by transmission electron microscopy (TEM) (Figure S1), due to different synthesis conditions (see section 1 of the Supporting Information). Accordingly, these nanoparticles

Figure 1. (a) Schematic diagram of the excitation pulse duration response of an upconversion system (red for excitation and green for upconversion luminescence). A 10% duty cycle is used for a better visual presentation. (b) Upconversion luminescence rise-decay profiles at 540 nm for the three Yb\(^{3+}\)-Er\(^{3+}\)-codoped upconversion nanoparticle samples with different kinetics (Figure S1) following a short-pulse excitation (10 \(\mu\)s) at 980 nm. (c) Upconversion luminescence spectra of the three Yb\(^{3+}\)-Er\(^{3+}\)-codoped upconversion nanoparticle samples with different kinetics (Figure S1) under continuous-wave 980 nm excitation. (d) Excitation pulse duration response of the 540 nm upconversion luminescence band for three Yb\(^{3+}\)-Er\(^{3+}\)-codoped upconversion nanoparticle samples with different kinetics (Figure S1), under excitation by a modulated 980 nm laser (0.2% duty cycle). Each data point is the average of three measurements. The standard deviation is much smaller than the symbol size. Measurements were performed at room temperature with an average excitation power density of 56 mW cm\(^{-2}\).
have different UCL kinetics under short-pulse 980 nm excitation, with the smallest UCNPs having faster kinetics and the larger core−shell UCNPs having slower kinetics (see Figure 1b and Figure S2). These three groups of nanoparticles are hereafter denoted as the “Fast”, “Medium”, and “Slow” groups, referring to their different UCL kinetics. They give typical UCL spectra of the Er\(^{3+}\) ions under continuous-wave 980 nm excitation (Figure 1c). Employing a simple lab-made setup (see details in section 2 of the Supporting Information), the excitation pulse duration responses of these UCNPs were measured by varying the excitation pulse width, while using a constant 0.2% duty cycle. The results for the Er\(^{3+}\) emission band at 540 nm are presented in Figure 1d. In the excitation pulse duration response curves, \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) generally exhibits a relatively fast increase in the short-pulse width range and then gradually reaches a plateau at longer pulse durations. Such a general trend can be explained by the fact that UCL originates from high-lying lanthanide excited states, which need time to be pumped, and thus, the upconversion emission system operates with a lower quantum efficiency at a non-steady state than at the steady state.\(^{15,21}\) It should be noted here that the selected 0.2% duty cycle was small enough to avoid pile-up effects (see the discussion in section S4 of the Supporting Information). It is noticeable in Figure 1d that the excitation pulse duration responses of the three different UCNPs samples show significant differences and that for UCNPs with fast kinetics a slight decrease in \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) can be noticed for longer values of \(w\) (>10 ms).

The excitation pulse duration response curves for other UCL bands of Er\(^{3+}\) ions of the three UCNPs samples were also observed, together with their impulse responses, as shown in Figures S2, S5, and S6. There were no significant pile-up effects found with a duty cycle of 0.2% (Figure S3). However, with a duty cycle of 2%, a significant pile-up was observed in all emission bands at shorter values of \(w\) (Figures S4 and S5).

To get a hold of which parameters can affect the UCL response to different excitation pulse durations, we performed numerical simulations based on a simplified two-photon upconversion model (Figure S7), representing a Yb\(^{3+}\) sensitized two-photon UCL emission. The model is described in detail in section S5 of the Supporting Information. The effects of the lifetime of the sensitizer excited state, the lifetime of the intermediate state of the activator ion, and the lifetime of the UCL emitting state of the activator ion, the energy transfer rates for the two energy transfer steps involved in the upconversion process, and the rate for a possible cross-relaxation process were investigated by respective simulations, while keeping other parameters constant. Small values for the duty cycle (Table S2) were selected to fully avoid the interference of the pile-up effect (Figure S8a). The calculated results show that the response in the modeled two-photon UCL, \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) is more sensitive to changes in the lifetime of the intermediate state of the activator ion and the energy transfer rates, and less to other parameters (Figure S8c–h). In particular, this response is very insensitive to the change in the lifetime of the UCL-emitting state (Figure S8e). This agrees with the results depicted in Figure 1d, where, given that the apparent decay times of the 540 nm band under short-pulse 980 nm excitation for the UCNPs with fast and medium kinetics are very different, they do not lead to very different excitation pulse duration response curves.

To gain more insight into the excitation pulse duration response of UCL to understand especially its insensitivity or sensitivity to different parameters, we performed in-depth theoretical analysis based on the rate equation model for a standard two-photon upconversion process (see details in sections S5 and S6 of the Supporting Information). By adapting a weak-excitation approximation to further simplify the question, we can describe the excitation pulse duration response by (sections S5 and S6 of the Supporting Information):

\[
\langle UCL(\lambda,w)\rangle_{\text{norm}} = C \left[ \frac{1}{k_{21}} - \frac{k_{21}^2}{k_{21}^2 - k_{51}^2} \left( e^{-k_{12}w} - 1 \right) \right]
\]

where \(C\) is a constant, \(k_{21}\) is the decay rate of the intermediate state of the activator (Er\(^{3+}\)\(^{1}I_{15/2}\)), and \(k_{51}\) is the effective decay rate of the excited state of the sensitizer (Yb\(^{3+}\)\(^{2}F_{5/2}\)), including the effect of the radiative decay rate (\(k_{4d}\)) and energy transfer rate (\(k_{c2}\)) (referencing to eq S5 of the Supporting Information).

Equation 2 indicates that the shape of \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) does not show dependence on the decay rate (or its inverse lifetime) of the UCL emitting state, agreeing well with the simulation result shown in Figure S8e. Equation 2 also predicts that \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) is largely determined by \(k_{21}\), the decay rate of the intermediate state of the activator (Er\(^{3+}\)\(^{1}I_{15/2}\)), completely in line with the simulation result shown in Figure S8d. In addition, the dependence of \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) on \(k_{51}\) the effective decay rate of the excited state of the sensitizer (Yb\(^{3+}\)\(^{2}F_{5/2}\)), can well explain the effects of the radiative decay rate (or its inverse lifetime) of the Yb\(^{3+}\)\(^{2}F_{5/2}\) state and the rate of transfer of energy from this state to the Er\(^{3+}\) ground state (\(\langle 1_{15/2}\rangle\)), while the effect of the latter is even larger than that of the former (Figure S8c,f), which can be explained by the difference in their magnitudes. Noting that typically \(k_{51} \gg k_{4d}\), according to eq 2, the shape of \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) may be dominantly affected by rate constant \(k_{21}\), it should also be noted that under the used weak-excitation approximation, i.e., neglecting the contribution of the second-step energy transfer process [from the Yb\(^{3+}\)\(^{2}F_{5/2}\) state to the Er\(^{3+}\)\(^{1}I_{15/2}\) state (ETU2 in Figure S7)] to the deactivation of the Yb\(^{3+}\)\(^{2}F_{5/2}\) and Er\(^{3+}\)\(^{1}I_{15/2}\) states, the effect of the rate for this process on \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) is not reflected in eq 2. Taken together, the excitation pulse duration response of UCL provides a facile approach to sense the information about the key intermediate states involved in the upconversion process and their population kinetics.

We point out that the limitation of our simulation studies and theoretical analysis, based on a model of the upconversion process, is obvious. In a real upconversion system, much more complicated processes could happen as disclosed in previous works, e.g., complex energy transfer and cross-relaxation pathways,\(^{22−24}\) surface coupling,\(^{25,26}\) and the thermal effect.\(^{27}\) These processes would more or less have an effect on the excitation pulse duration response of the UCL and accordingly change the information that we can get from this response. For instance, in the simulations, we could not reproduce the decrease in \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) for longer values of \(w\) (>10 ms), as experimentally found in the case of the 540 nm UCL band of the “Fast” group of UCNPs (Figure 1d). With regard to this specific behavior, we speculate that it was due to the ignition of the higher-order energy transfer upconversion process reinforced by a long excitation pulse duration, similar to that reported in a previous work.\(^{18}\)
Here, it is instructive to further discuss the possibility of extracting the important parameter values characterizing the UCL from the experimental excitation pulse duration response data. For comparison, in TRAST spectroscopy studies, the same modulation-detection approach has been successfully applied to quantify dark state transitions in organic fluorophores for bioimaging and biosensing purposes.\textsuperscript{17,26,29} While our experimental data show that this approach is also feasible for UCNPs, the larger number of states involved and the nonlinear response of UCNPs significantly increase the model complexity and add difficulty in retrieving the rate parameter values. On the contrary, a consequence of this added complexity is also that the excitation pulse duration response of UCNPs can be used as an optical encoding/decoding technique for encryption applications.\textsuperscript{30,31} It allows the possibility of information being encrypted within different groups of UCNPs showing the same emission color (upon NIR excitation) but having different excitation pulse duration responses. Distinguishing different UCNPs by their responses to excitation pulse durations thus opens an almost infinite internal encoding space. In addition, because our analysis requires that only the time-averaged UCL intensity \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) needs to be detected, multipixel detectors with low time resolution can be used for decoding. This strongly reduces the instrumental requirements, increases applicability, and makes possible a faster reading than when point-like detectors must be used.

It is known that the luminescence kinetics of UCNPs exhibit a significant dependence on the excitation intensity.\textsuperscript{32–34} A high excitation intensity favors the populations of high-lying energy levels and can lead to significantly different intensity ratios between different emission bands compared to under low-intensity excitation. We have studied the effect of excitation intensity on the excitation pulse duration response of UCL. Figure 2a shows how \(\langle UCL(\lambda,w)\rangle_{\text{norm}}\) at \(\lambda = 540\) nm for the Yb\textsuperscript{3+}-Er\textsuperscript{3+}-codoped UCNPs with slow kinetics varies with \(w\), and how the average excitation intensity, \(I_{\text{exc}}\), influences the excitation pulse duration response curves significantly, where a higher \(I_{\text{exc}}\) generally increased the UCL intensity at all values of \(w\) and reduced the variation ranges of the response curves. This trend could also be reproduced by numerical simulations (Figure S8b). Further analyzing the \(I_{\text{exc}}\) dependence in the excitation pulse duration response curves recorded from the 540 nm UCL band (Figure 2b) revealed that the slope of the UCL versus \(I_{\text{exc}}\) increases with a decrease in pulse width, which is in large contrast to the corresponding dependence under continuous-wave excitation (Figure 2c). Similar effects caused by variation of \(I_{\text{exc}}\) were also found for other UCL bands (Figure S9). This implies that the use of short excitation pulses better preserves the nonlinear optical responses of UCNPs and may be particularly useful for optical sectioning and super-resolution microscopy techniques, which specifically benefit from the nonlinearity of the UCNPs.\textsuperscript{35–38}

We further examined the response in \(\langle UCL(\lambda,w)\rangle\) to excitation pulse duration \(w\) over the full UCL spectrum (400–900 nm) of the Yb\textsuperscript{3+}-Er\textsuperscript{3+}-codoped UCNPs with slow kinetics. The UCL spectrum of this UCNP sample shows a noticeable difference under pulsed excitation with different pulse widths (Figure 3b). The different UCL emission bands can be assigned to transitions of Er\textsuperscript{3+} ions shown in Figure 3a. It is known that different emission bands from the same UCNPs may have distinct or the same kinetics, depending on if they refer to a transition originating from a different or the
same higher energy level(s), respectively. Because the excitation pulse duration response is closely related to UCL kinetics, we propose that it can be used to identify the emission peaks originating from the same higher-energy state. For instance, the emission peaks at 540 and 840 nm both originate from the same $^4S_{3/2}$ energy state (Figure 3a), and thus, they exhibit almost identical excitation pulse duration responses (Figure 3c). In addition, the 410 and 555 nm emission peaks, originating from transitions from the same $^2H_{9/2}$ energy level, also show very similar excitation pulse duration responses (Figure 3c). Interestingly, the 520 and 540 nm emissions, well known to originate from transitions from two strongly thermally coupled states ($^2H_{11/2}$ and $^2S_{3/2}$), show noticeable differences in their excitation pulse duration response curves. These differences could be a result of the differences in the coupling channels of these two states with other Yb$^{3+}$ or Er$^{3+}$ states. For example, it was reported that the Er$^{3+}$ $^4S_{3/2}$ state could be involved in a cross-relaxation process between two Er$^{3+}$ ions ($^4S_{3/2} + ^4I_{15/2} \rightarrow ^4I_{13/2} + ^4I_{11/2}$)\(^{40}\), an energy transfer process involving Er$^{3+}$ and Yb$^{3+}$ ($^4S_{3/2}$ (Er$^{3+}$) + $^2F_{7/2}$ (Yb$^{3+}$) $\rightarrow$ $^4I_{13/2}$ (Er$^{3+}$) + $^2F_{5/2}$ (Yb$^{3+}$))\(^{41}\), and an excited state absorption process of Er$^{3+}$ ($^4S_{3/2}$ $\rightarrow$ $^2F_{5/2}$)\(^{40}\), but not the Er$^{3+}$ $^2H_{11/2}$ state. This requires further investigation.

We also performed some excitation pulse duration response studies of NaYF$_4$:Yb$^{3+}$:Er$^{3+}$ UCNPs, with multiple well-assigned UCL bands, some of which are known to originate from transitions from the same higher state (Figure S13). An appropriate duty cycle was used for each emission band to avoid pile-up effects (Figure S13i). Similar to the case of Er$^{3+}$-based UCNPs, the emission peaks from transitions from the same higher state, e.g., the 475 and 645 nm peaks from the $^4G_4$ energy level and the 360, 450, and 740 nm peaks from the $^4D_2$ energy level, exhibit very similar excitation pulse duration response curves (Figure S13j).

This observation made us realize that the distinct excitation pulse duration responses of spectrally overlapping emission peaks are actually coming from different transitions and indicate the possibility of suppressing a certain emission peak by using a proper square-wave excitation pulse width. This may have important implications for biosensing applications of UCNPs. One example shown here is given by the two green emission peaks at 540 and 555 nm, originating from the $^4S_{3/2}$ $\rightarrow$ $^4I_{15/2}$ and $^2H_{9/2}$ $\rightarrow$ $^4I_{13/2}$ transitions, respectively (Figure 3a). As indicated by their distinct excitation pulse duration response curves (Figure 3c), it is predictable that the emission peak at 555 nm can be suppressed while still exciting the main emission band at 540 nm, if the UCNPs are excited by square-wave excitation with proper parameters (see Figures 3b and 4a,b). Because the 555 nm emission peak comes from a transition from a higher-lying energy level, its intensity usually increases faster with an increase in $I_{exc}$ than the $^4S_{3/2}$ emission. However, we found that the effectiveness of using the short-pulse excitation approach to suppress the intensity of the 555 nm emission peak relative to the 540 nm main band remains in a broad range of average excitation densities, 12–183 mW cm$^{-2}$ (the limits of our instrument), as shown in Figure S12a. Nevertheless, the suppression of the contribution of the 555 nm emission peak to the main green emission band may have a slightly reduced effectiveness if the duty cycle of the modulated excitation is not sufficiently low to avoid pile-up effects (Figure S12c,d).

The possibility of suppressing the emission peak at 555 nm of Er$^{3+}$ ions is of high interest for nanothermometry applications using Yb$^{3+}$-Er$^{3+}$-codoped UCNPs, where the intensity ratio between the sub-bands at 520 ($^2H_{11/2}$ $\rightarrow$ $^4I_{15/2}$) and 540 nm ($^4S_{3/2}$ $\rightarrow$ $^4I_{15/2}$) is frequently used as the temperature indicator. The principle is that the populations at
standard deviation is much smaller than the symbol size. Measured at room temperature with an average excitation power density of 56 mW cm\(^{-2}\). (c) Fitted energy mismatch (\(\Delta E\)) between the \(^{2}H_{11/2}\) and \(^{4}S_{5/2}\) energy levels of Er\(^{3+}\) ions using intensity ratio data measured under 980 nm continuous-wave and pulsed excitations, including (red) or excluding (green) the 555 nm emission peak in the integrated area. Error bars were obtained from the 95% confidence interval of \(\Delta E\) in the fitting to the Boltzmann distribution \(I_{520}/I_{540} \propto \exp(-\Delta E/k_BT)\). The average value of \(R^2\) is 0.987 ± 0.004. See more details in section S8 of the Supporting Information.

Lanthanide-doped upconversion nanoparticles (UCNPs), operating as optical kinetic systems, exhibit fascinating features in response to temporal excitation modulations. As particularly studied in this work, the response of the upconversion luminescence of UCNPs to the pulse width in square-wave excitation shows a complex dependence on the fundamental photophysical parameters characterizing the upconversion mechanisms and on the excitation intensity. As implicated by the results presented in this work, the square-wave excitation modulation technique offers many opportunities for investigating and applying UCNPs, e.g., for anticounterfeiting, identifying emission peaks originating from transitions from the same excited state, super-resolution microscopy, and reliable luminescence nanothermometry.

\section*{ASSOCIATED CONTENT}

\section*{Supporting Information}

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.2c03037.

Additional experimental details, materials and methods, and supporting data on characterization of nanomateri-
als, effect of the duty cycle, rate equation simulations, theoretical analysis, effect of excitation intensity, effect of temperature, Er³⁺ emission band ratios, and ratio to Yb³⁺-Tm³⁺-codoped nanoparticles (PDF)
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