# **Electronic Supplementary Information (ESI)**

### 1 Measurement procedure

### 1.1 Reference dye absolute $\phi$ measurement

The photoluminescence  $\phi$  was determined absolutely with the custom build integrating sphere setup as previously described and addressed in the main article. The reference dye DY-781 was excited at 710 nm. The absorption of the sample in the integrating sphere at 785 nm was (33.8  $\pm$  0.2) % and therefore at a similar concentration as in the upconversion  $\phi$  measurements. The uncertainties are represented by the standard deviation of 6 separate measurements.

#### **1.2** Reference dye relative $\phi$ measurement

Water and ethanol were used to clean out two cuvettes (10 mm  $\times$ 10 mm). The cuvettes were then dried until there was no residual solvent present. One cuvette was marked as the sample cuvette and was filled with 2.0 ml 25 nanomolar DY-781 diluted in ethanol. The second cuvette was labelled as the blank cuvette and was filled with 2.0 ml ethanol. A 785 nm laser diode was used to excite the reference dye DY-781; the wavelength 785 nm closely matches the maximum absorption peak of DY-781. The laser temperature controller and laser driver were turned on, and the temperature set to 25 °C. The measurement time was set to 200 s, with a sample rate of 25.0 kHz for the APD and 1.0 Hz for the power meter. The blank cuvette was first placed into the cuvette holder, and the laser driver current was adjusted to provide the drive current needed for the first measure data point. The APD and the power meter read out programs were started simultaneously. After 65 s the blank cuvette was quickly removed from the system and the system recorded the background (empty cuvette holder) for another 65 s. The sample cuvette was then placed in the cuvette holder, right after the background measurement was finished and the system was recording continuously until both programs reached their measurement time limit of 200 s. The procedure was repeated with increasing excitation light power for all data points until reaching the laser driver power limit. In this measurement series the excitation power density was increased with a multiplication factor of approximately 1.4 between first and last data points being 0.01 Wcm<sup>-2</sup> and 2 Wcm<sup>-2</sup>, respectively. (the beam area was assumed to be  $5.0*10^{-3}$  cm<sup>2</sup> corresponding to a beam width of 0.076 cm).

### **1.3** UCNPs relative $\phi$ measurement

Two cuvettes were cleaned by using the same method as used in section 1.2. If the cuvette had contained fluorophores before, hydrogen chloride was used to remove any possibly remaining fluorophore inside the cuvette. The cuvette was filled with hydrogen chloride for at least one hour, after which the hydrogen chloride was poured out, and the cuvette was cleaned with water and ethanol. No residual solvent was presented after the cuvette was dried out.

The blank cuvette was filled with 2.0 ml cyclohexane. The sample cuvette was filled with 2.0 ml of the UCNPs dispersion. For these measurements the 975 nm laser was employed. The laser driver current was adjusted to the first measured data point of 0.2 Wcm<sup>-2</sup> (the beam area was assumed to be 5.0\*10<sup>-3</sup> cm<sup>2</sup>). The cyclohexane blank sample was placed in the cuvette holder and the APD and power meter read out programs were started simultaneously. The same procedure as for the reference dye was employed to collect the data. The procedure was repeated for multiple data points until the laser reached its maximum power of 0.15 W (corresponding to a power density of 30  $Wcm^{-2}$  in the focus). The data points follow again a power density increase by a factor of approximately 1.4 between measurements. The most significant difference in these two procedures is recording the beam profile image after each measurement using the mounted CCD camera. At low excitation power the beam profile can be recorded readily while at high excitation power the ND step filters needed to be placed in front of the camera to avoid saturation.

# 2 Raw data

Figure S1a presents the raw data collected from power meter when data regarding the UCNPs was recorded. The high power values in the middle of the figure are recorded when the sample was removed from the cuvette holder. Thus, it indicates the laser excitation power ( $P_o$ ). When sample cuvette containing the UCNPs is placed at cuvette holder the power has lowest value (Counts between almost 140 and 200) representing the absorption caused by nanoparticles. The counts between 0 to 65 are data collected when the cyclohexane was placed in cuvette holder. Similar raw data is acquired from APDs simultaneously as shown in Fig. S1b. No signal is detected when the cyclohexane is placed (Counts between 0 and 65) and when the cuvette holder is empty (Counts between 65 and 130). The absorption coefficient value for UCNPs with 0.85% variation and for Dy-781 with 0.88% were calculated.



**Fig. S1** The recorded a) transmitted signal and b) luminescence signal of UCNPs. The transmitted signal is recorded via power meter when three different cuvettes are placed in cuvette holder. The luminescence signal is detected by APD while laser power varies.



**Fig. S2** a) Gaussian beam profile and b) top hat profile with same total power and a diameter equal to the FWHM of Gaussian profile. c) and d) the same profiles squared

# 3 Beam profile dependence simulations

The beam diameter of a top hat beam is chosen as the FWHM the Gaussian beam. The same total excitation power is used the two models, meaning that the volumes under the surfaces f the two cases are the same, see Figs S2a, SS2b and SS3a. How ever, the UCNPs luminescence signal intensity (*L*) is not linear dependent on the excitation power density ( $\rho_{ex}$ ) but exhibits power dependence for low power densities described as

$$L \propto \rho_{ex}^p$$
, (S1)

where p denotes the order of the upconversion process, i.e. how many photons are involved in each excitation process. In our model, considering the two-photon process for the Tm emission at 800 nm, p = 2 for a low excitation power. For the sake of argument let us first for simplicity assume that all power densities are low, so that this squared relation is valid both in the centre of the beam and on its flanks. Higher powers will break this assumption, where saturation first will influence the centre of the beam, and for even higher power also increasingly much the flanks. With the assumptions, the emission signal will scale as the square of the excitation power density, illustrated in Figs S2c, SS2d and SS3b. This simple model gives that the volume under the surfaces now differ by a factor of 0.66 and 0.44 for the 1D and 2D models, respectively, illustrating that the luminescence indeed is sensitive to the beam profile, even in the simple case of nonsaturation. The issue becomes quickly more complex once part of the beam is deviating from a squared power dependence due to saturation tendencies in the excitation process.



**Fig. S3** Comparison of a) Gaussian and top hat beam profile cross sections and b) Gaussian and top hat squared beam profile cross sections.

# 4 Filters effect

The NaYF<sub>4</sub>:Yb<sup>3+</sup>,Tm<sup>3+</sup> particles emit the major UC emission at around 800 nm under excitation of 975 nm light. The bandpass filter used in the detection arm of UCNPs is centered at 800 nm with FWHM=50 and 80% transmission. For the reference dye, the emission spectra of DY-781 is broader than filter used in the detection arm. The DY-781 exhibits a  $\phi$  of 12.4% when dissolved in spectroscopic grade ethanol. Using two filters in front of the luminescence reduce this value significantly since a small part of this luminescence is reaching APD2. These reductions in luminescence signals can be calculated by looking at the area under the emission spectra as shown in Fig. S4 and integrating the emission intensity and therefore considered in  $\phi$  value calculation. Thereby, integrated transmission of the wavelength filters  $T = \int T_{(\lambda)} \times S_{(\lambda)} d\lambda$ , where  $S_{(\lambda)}$  is the area normalized corrected emission spectrum.



Fig. S4 The normalized UCNPs and Standard dye spectra profile are shown. The 800nm bandpass filter has maximum 80% transmission with FWHM of 50 nm, while the 830 nm filter set has a maximum transmisson of 20% and a FWHM of 10 nm.

# 5 Scattering compensation

In evaluating  $\phi$  of UCNPs, the absorption measurements conducted needs to be compensated for any light scattering attenuation. To obtain this information, the UCNPs was irradiated by a white light source and the attenuation was measured as a function of wavelength. The blue curve in Fig. S5 shows the spectrum obtained from this measurement and it exhibits significant sign of scattering, especially at the shorter wavelengths. Any scattering is assumed to be due to Rayleigh scattering in the sample, and a the red curve is a fit with a  $1/\lambda^{-4}$  dependence. By subsequently subtracting the extrapolated scattering plot from the blue curve, a new curve is obtained that represents attenuation coefficient due to pure absorption (black curve). The ratio between black and blue curve at any specific wavelength indicates the influence of scattering. In this particular experiment, the influence of the scattering at the evaluated absorption wavelength is 6.7%.



Fig. S5 The influence of scattering on UCNPs luminescence materials during quantum yield measurement.

# 6 Fitting procedure

In calculating  $\phi$  using Eq. 4 (blue curve in Fig. 4), we have assumed that the intensity of the excitation radiation is uniform across the beam-profile and that, as a result, all UCNPs exhibit the same  $\phi$  for each data point. Explicitly the value on the x-axis  $\rho_k$  is for each data point *k* and each associated  $\phi$  value  $\phi_k$  on the y-axis derived using Eq. S2.

$$\rho_k = \frac{P_k}{\pi (\frac{w_k}{2})^2},\tag{S2}$$

Using the  $\rho_k$  values to describe the fundamental relationship between excitation power density and  $\phi$  however, is non-ideal. Since the choice of what beam diameter definition to use for  $w_k$ is arbitrary (FWHM,  $1/e^2$ -width etc.) the  $\rho_k$  values are to some extent arbitrary. The exact values of  $w_k$  might also be difficult to reproduce over different measurement sessions and for other research groups. More significantly, it is straightforward to show that knowing the quantity  $\rho_k$  is on its own not enough to predict a unique  $\phi$  value. Our suggestion for describing the relationship between the UCNPs  $\phi$  and the excitation power density in a better way, using the existing measurement data, is to view each beam profile as a collection of top-hat profiles. Consider the situation when each beam profile is discretized into an  $m \times m$  grid across the 2D beam profile. With m large enough, each grid zone can be seen as having a constant excitation power density. The total luminescence signal is thus seen as a sum of the contributions from  $m^2$  zones over which the  $\phi$  is locally constant. Crucially, these  $\phi$  values,  $\phi_{uniform}$  represent the upconversion efficiency of the UCNPs under a uniform excitation power density and are as a result only affected by the physical characteristics of the nanoparticles and not by measurement specific conditions such as the beam diameter.

Mathematically the entire measurement session can be described using equation system,

$$\sum_{i=1}^{m} \sum_{j=1}^{m} a_{(i,j,k)} \cdot \phi_{uniform}(y_{(i,j,k)}) = \phi_k(\rho_k), \quad k = 1...n$$
(S3)

where the weighting factors  $a_{(i,j,k)}$  are given by

$$a_{(i,j,k)} = \frac{y_{(i,j,k)}}{p_k}$$
 (S4)

and account for the fact that the number of upconversion processes taking place in each grid zone (i, j, k) is related to the local uniform excitation power  $y_{(i,j,k)}$  in that zone. In this equation  $\phi_{uniform}$  will fulfil Eq. 1 and  $\phi_k$  is the experimentally measured quantum yield. Note that the total sum of the  $m^2 y_{(i,j,k)}$  values is equal to  $P_k$  for each k. Each k represents a data point (black star) in Fig. 4. In our case n=16. Each summation term of the LHS corresponds to one grid zone with a constant excitation power density and as a result a constant  $\phi$ . The RHS meanwhile, is made up of the measured  $\phi$  values included in Fig. 4. As a final step we assume that  $\phi_{uniform}$  can be accurately modelled using the relationship described in Eq. 1 with some fitting parameters  $\phi_b = \phi_c$  and  $\rho_b = \rho_c$ . The excitation power density value associated with each grid zone (i, j, k) can be estimated in two different ways using the  $y_{(i,j,k)}$  values. Method 1 assumes the excitation profiles are perfectly Gaussian, with  $P_k$  and  $w_k$  characterizing the profiles fully, whereas Method 2 utilizes the actual beam profile images to perform the corresponding calculations. Since  $y_{(i,j,k)}$ ,  $P_k$  and  $\phi_k$  are determined experimentally, this is an overdetermined nonlinear equation system with only two unknowns,  $\phi_c$  and  $\rho_c$ . The red and the green curves in Fig. 4 are determined in MatLab by fitting  $\phi_c$  and  $\rho_c$  to the available data using the lsqnonlin nonlinear fitting algorithm.