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Supplementary Information

Intrinsic optical sectioning with upconverting

nanoparticles.



Figure ESI1: Top: Emission of the used UCNPs under irradiation at 980 nm. Bottom: states diagram of the main emission processes.



Figure ESI2: Top: Irradiation of the 980 nm laser (ON-OFF). Bottom: Time-resolved emission of the used UCNPs below 750 nm.



Figure ESI3: Overall scheme of the SLUM. A NIR (980 nm) solid state 10 mW laser (a) is coupled to a single mode fiber optics (b), collimated with plano convex lens and sent using two mirrors (d,e) to a pair of X-Y scan galvos (f,g) which are confocal through a telescope h. The scanned NIR beam is enlarged with a second telescope (i), passes through a dichroic mirror (j) and directed to the objective (k) to the specimen whose z-position can be modified with a speaker (m). The emission of the specimen ($\lambda < 750$ nm) is collected from the objective and reflected by the dichroic mirror, passes through a block filter

that removes any residual NIR excitation (n) and is focused through a lens tube (o) onto the CCD sensor (p).

Sequential scanning and parallel collection

In order to minimize the time required for imaging, SLUM utilizes parallel rather than sequential collection of the sample emission. The scanning is conventional as depicted in figure ESI4.



Figure ESI4: Scheme of the scanning procedure with typical parameters (see text).

A focused spot of 980 nm laser sweeps the imaging plane at a given speed. The dwell time of the spot at a given emitter is much less than the characteristic times of the photophysical processes involved. This brief excitation avoids saturating the intermediate states of the UCNPs individual emitter. However, the whole image receives enough excitation to produce a rather bright image which has to be collected and descanned. This descanned collection is solved by focusing the emission directly on a CCD sensor, which collects in each of its pixels all the light that each point in the specimen has emitted during the frame acquisition time.



Figure ESI5: Comparison of SLUM images taken at different excitation powers. a) Bright field images of a pollen grain illuminated with a 525 nm LED source. b) Linear epi-illumination images of the same specimen using a non focused 980nm Laser Diode excitation. c-e) SLUM images at excitation power

p = 3 mW, p = 1 mW and $p = 500 \mu \text{W}$ respectively. Columns c and d show the effect of a diminished

z-sectioning due to saturation (scanning speed = 19.2 cm/s).

Synthesis

All reagents were commercially available and used as received.

β-NaYF₄: 2 mol% Er³⁺, 30 mol% Yb³⁺ core nanoparticles. In a typical synthesis, Y(CH₃CO₂)₃•xH₂O (186 mg, 0.7 mmol), Yb(CH₃CO₂)₃•xH₂O (105 mg, 0.3 mmol) and Er(CH₃CO₂)₃•xH₂O (7 mg, 0.02 mmol) were added to a 50 mL threeneck round-bottom flask containing octadecene (15 mL) and oleic acid (6 mL). The solution was stirred magnetically and heated slowly to 120 °C under vacuum for 30 min to form the lanthanide oleate complexes, and to remove residual water and oxygen. The temperature was then lowered to 50 °C and the reaction flask placed under a gentle flow of nitrogen gas. During this time, a solution of ammonium fluoride (148 mg, 4 mmol) and sodium hydroxide (100 mg, 2.5 mmol) dissolved in methanol (10 mL) by stirring. Once the reaction reached 50 °C, the methanol solution was added to the reaction flask and the resulting cloudy mixture was stirred for 30 min at 50 °C. The reaction temperature was then increased to 70 °C and the methanol evaporated from the reaction mixture. Subsequently, the reaction temperature was increased to 300 °C as quickly as possible by means of a aluminum heat conduction piece placed between the heater plate and the flask. The temperature was maintained for 90 min under the nitrogen gas flow. During this time the reaction mixture became progressively clearer until a completely clear, slightly yellowish solution was obtained. The mixture was allowed to cool to room temperature. The nanoparticles were precipitated by the addition of ethanol and isolated via centrifugation at 5000 rpm. The resulting pellet was dispersed in a minimal amount of Hexane(2.5ml) and precipitated with excess ethanol. The nanoparticles were isolated via centrifugation at 4500 rpm and then dispersed in hexanes (5 mL) for the subsequent shell growth procedure.

β-NaYF₄: 2 mol% Er³⁺, 30 mol% Yb³⁺ / β-NaYF₄ core-shell nanoparticles. Y(CH₃CO₂)₃•xH₂O (240 mg, 0.9 mmol) was added to a 50 mL threeneck round-bottom flask containing octadecene (15 mL) and oleic acid (6 mL). The solution was heated slowly to 120 °C under vacuum with magnetic stirring for 30 min. The temperature was lowered to 80 °C, the reaction flask placed under a gentle flow of nitrogen and the dispersion of NaYF₄: 2 mol% Er³⁺, 30 mol% Yb³⁺ core nanoparticles in hexanes was added. The resulting solution was slowly heated to 110 °C and maintained at this temperature until all the hexanes were removed. The reaction mixturewas cooled to 50 °C and a solution of ammonium fluoride (130 mg, 3.5 mmol) and sodium hydroxide (85 mg, 2.2 mmol) dissolved in methanol (10 mL) was added. The resulting cloudy mixture was stirred for 30 min at 50 °C at which time the reaction temperature was increased to 300 °C as quickly as possible by means of a aluminum heat conduction piece placed between the heater plate and the flask and maintained at this temperature for 90 minutes under the nitrogen gas flow. The mixture was allowed to cool to room temperature and the nanoparticles isolated using the same procedure of precipitate and isolation described for the core nanoparticles. The isolated NaYF4:Er Yb nanoparticles were dispersed in cyclohexane (~7 mL) for subsequent experiments.

Typical UCNP produced by this procedure are monodisperse and elongated about 40 nm long and 20 nm wide as depicted in Figure ESI6. Their photophysical parameters are given in Fig ESI1 and ESI2.



Figure ESI6: SEM images of typical UCNPs obtained through the described synthesis procedure.

Methods

The UV-Vis spectra were taken with an Ocean Optics CHEM2000 diode-array spectrometer. A TO-18 (5.6 mm diameter, LCU985041A), 50 mW, 980 nm laser diode was used as excitation source.

A QPhotonics Single mode fiber coupled laser diode QFLD-980-10SAX, p=10 mW λ = 980 nm was used as SLUM excitation source. The laser beam from the fiber tip was collimated using a 25 mm focal distance lens to enter the telescope (see figure S3).

A PointGrey Flea 2 monochrome CCD camera (FL2-03S2M-C) was used as sensor.

The traces of the time resolved emission were acquired with a Keysight DSO1052B digital oscilloscope and a Thorlabs PDA36A Si Transimpedance Amplified Photodetector placed instead of the CCD camera.

Derivation of Equation (1)

For a Gaussian beam focused onto a sample, the lateral distribution of intensity at the focal plane can be described as:

$$I(r) = I_0 \exp\left(-\frac{2r^2}{\omega_0^2}\right) \tag{1}$$

where I_0 is the intensity at the center of the distribution, r is the distance from the propagation axis and ω_0 , the Gaussian beam waist, is the distance at which the intensity has decreased to $1/e^2 \approx 0.135$.

The waist ω_0 is related with the wavelength λ and the angular aperture θ :

$$\omega_0 = \frac{\lambda}{\pi \,\theta} \tag{2}$$

On the propagation axis, the size of the waist increases below and above the focal plane:

$$\omega(z) = \omega_0 \sqrt{1 + \left(\frac{z \text{ NA}}{\omega_0}\right)^2} \qquad (3)$$

where NA = n sin θ is the numerical aperture of the objective (n = refractive index of the sample). Therefore, the equivalent area of the illuminated zone at any distance *z* from the focus is:

$$A(z) = \pi \left(\omega_0^2 + z^2 \mathrm{NA}^2 \right)$$
 (4)

For a given photon flux and in absence of absorption, equation (4) represents the reciprocal of the excitation density. For a laterally infinite and homogeneous emissive thin layer which is scanned in x and y directions, the total collected light does not depend on the focusing, but only on the emission intensity of the layer. Therefore, if this emission is due to a linear process, no change will be apparent by changing z-position. However, for a nonlinear process, the collected emission of the layer will be a function of the position as follows:

$$I_{em} = k (A(z))^{1-n} = k \pi (\omega_0^2 + z^2 N A^2)^{-n}$$

where k is a constant that includes all instrumental parameters and also the emission quantum yield of the probe, and n is the number of photons of the nonlinear process.