

**Figure 2** | Experimental results and theoretical calculations for a tightly focussed, radially polarized vector beam. **a,b**, Colour-scale representations of the experimentally reconstructed distributions of  $|E_x|^2$  (**a**) and  $\Phi_x$  (**b**). Two lobes with a lateral size of roughly 250 nm are easily resolved. The phase image shows that the left-hand side of the focus is out of phase with respect to the right-hand side. **c**, Colour-scale representation of the distributions of  $|E_x|^2$  and  $\Phi_x$  as calculated by vector diffraction theory.

with a spatial intensity distribution whose typical feature size is of the order of 250 nm. Figure 2 shows both the reconstructed distribution of  $|E_x|^2$  and  $\Phi_x$  and the same entities as calculated by vector diffraction theory. It is immediately apparent from this figure that excellent agreement is obtained between the measurement and reconstruction on the one hand and the fields calculated by diffraction theory. The power of the technique, however, is exemplified by the small (subwavelength) deviations between the measurement and the theory. A detailed analysis of the results shows that these subtle deviations, rather than showing the limits of the technique, indicate minute imperfections in the focusing system.

It is precisely this ability that is anticipated to aid advances in modern optical microscopy. Such advances rely on using combinations of multiple foci

and nonlinear interactions, such as saturation. Advancing optical microscopy therefore requires a detailed knowledge of the complex optical field distributions at the focus, which can now be easily characterized. This will facilitate data interpretation, help optimize the resolution and aid the development of new microscopy schemes. The method can also be used to map other (nano)structured light fields with phase sensitivity, so long as a propagating far-field reference can be provided. It may allow the characterization of near-field probes if rescattering of light from the probe can be neglected. Mapping the full vector field of light will also be crucial for exploring novel knotted solutions of Maxwell's equations<sup>9</sup>. Ultimately, if the original objection of Einstein to Sygne's scheme, namely having a large substrate close to (and evanescently coupled to) the

sample, can be overcome, the method could be used to realize quantitative microscopy of confined fields. □

*L. Kuipers is at the Center for Nanophotonics, FOM Institute AMOLF, Science Park 104, 1098 XG, Amsterdam, The Netherlands.  
e-mail: l.kuipers@amolf.nl*

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## OPTICAL MULTIPLEXING

# Tunable lifetime nanocrystals

Tuning the luminescence lifetimes of upconversion nanocrystals through lanthanide doping provides new opportunities for optical multiplexing in the time domain for applications in imaging and security marking.

Renren Deng and Xiaogang Liu

The ability to simultaneously image multiple features or events of interest through the use of luminescent materials is essential to a myriad of modern technologies. Applications of these light-emitting materials range from biological assay and molecular sensing to data storage and document security<sup>1,2</sup>. Optical multiplexing in biologically relevant

settings is typically achieved by employing several organic dyes or luminescent nanocrystals with distinct emission wavelengths as markers. Different features or events are then simply distinguished by their emission colour<sup>1,3</sup>. However, the significant spectral overlap of commonly used luminescent probes limits the degree of multiplexing, and hence

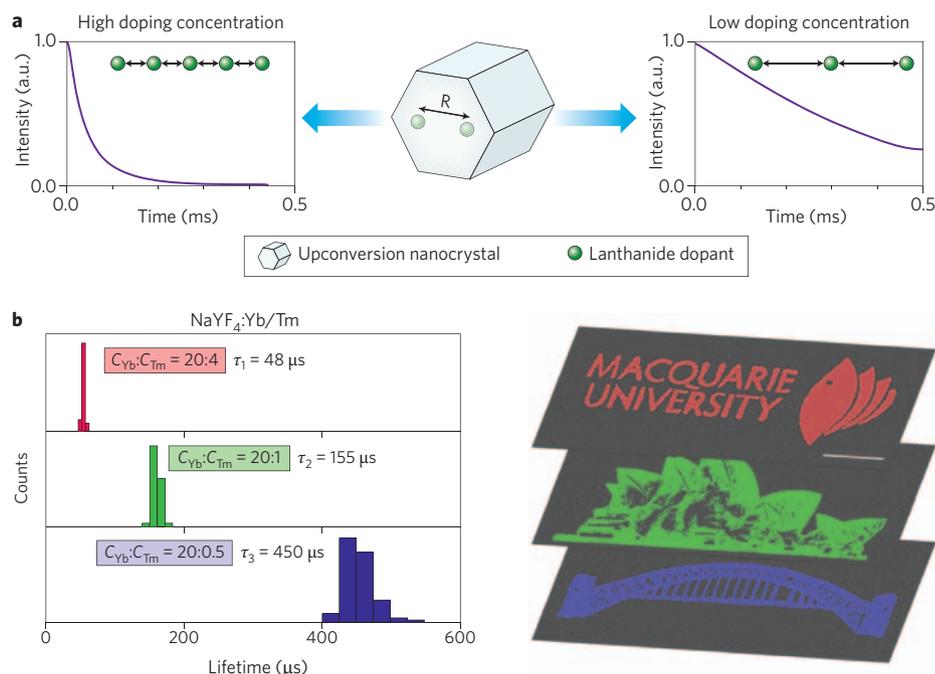
the number of probes that can be employed in practice. Alternatively, the different lifetime decay rates of various optical probes can be utilized as a new dimension for multiplexing<sup>4</sup>, allowing probes to be distinguished by the temporal characteristics of their light emission. This lifetime-based separation technique can dramatically increase

the opportunities for multiplexing, but it requires the development of suitable probes whose lifetimes can be tuned and that can be excited by a single, common wavelength.

Now, writing in *Nature Photonics*, Yiqing Lu *et al.* demonstrate the possibility of generating a library of distinct time-domain codes, specially designed for a single emission band, using lanthanide-doped upconversion nanocrystals<sup>5</sup>. All upconversion nanocrystals have three general properties: specific, line-like emission peaks, intrinsically long emission lifetimes and the remarkable ability of combining two low-energy photons to produce a single high-energy photon — a useful nonlinear optical process known as upconversion<sup>6</sup>. In contrast to conventional organic dyes and semiconductor quantum dots, which both possess very short lifetimes (typically 0.1–20 ns), upconversion nanocrystals exhibit much longer decay times of the order of microseconds. With a time-gated detector, the long luminescence lifetimes of the nanocrystals can be easily separated from the short-lived autofluorescence — the natural emission of light by biological specimens on excitation, which usually takes place on the order of several nanoseconds<sup>7</sup>. However, there is the issue of how best to control and fine-tune the lifetimes that cover a broad temporal range, as the luminescence kinetics of upconversion nanocrystals are generally influenced by many factors, including particle shape, size, phase and surface ligand passivation as well as dopant concentration.

Lu and colleagues recognized that the spectral properties of upconversion nanocrystals are highly sensitive to the rate of energy transfer between the lanthanide ions doped in the nanocrystals. The occurrence of a fast energy migration process is likely to induce quick dissipation of the excitation energy, resulting in a reduction in the emission lifetime of the nanocrystals. This means that precise control over the luminescence kinetics can be achieved by varying the amount of doping based on a knowledge of concentration quenching<sup>8</sup>. In principle, the lifetimes of the excited states of heavily doped nanoparticles are expected to be short owing to increased rates of energy migration with reduced ionic distances (Fig. 1a).

As a test case, the researchers used thulium-doped upconversion nanoparticles with ytterbium sensitizers (NaYF<sub>4</sub>:Yb/Tm). The Yb<sup>3+</sup> sensitizer is used because it



**Figure 1** | Luminescence lifetime multiplexing through lanthanide doping in upconversion nanocrystals.

**a**, Schematic illustration and luminescence decay studies showing the lifetime tuning of NaYF<sub>4</sub>:Yb/Tm nanocrystals. The luminescence lifetime of the nanocrystals can be tuned by adjusting the doping concentration, allowing precise control over the average ionic distance ( $R$ ) between the trivalent lanthanide dopants. **b**, Demonstration of security printing and optical information storage using lifetime-encoded upconversion nanocrystals. Three overlaid patterns printed with nanocrystals having three different lifetimes ( $\tau_1$ ,  $\tau_2$ ,  $\tau_3$ ) can be decoded and separately read out by a time-resolved confocal fluorescence microscope equipped with a 980-nm laser. The different lifetimes of the nanocrystals were generated by controlling the concentration ratio of Yb<sup>3+</sup> and Tm<sup>3+</sup> ( $C_{Yb}:C_{Tm}$ ). Note that the emission colour is the same in all cases; the red, green and purple colours in the figure indicate the different lifetimes.

strongly absorbs light in the near-infrared region, and can thus be conveniently excited by popular 980-nm laser systems. The researchers tweaked the concentration of Tm<sup>3+</sup> activator in the range 0.2–8 mol% to tune the lifetimes. Their technique proved highly effective, yielding broadly tunable lifetimes from 25.6  $\mu$ s to 662.4  $\mu$ s in the blue emission band of Tm<sup>3+</sup> at around 475 nm. Through experiments involving the optimization of both doping concentration and composition, the researchers succeeded in preparing eight sets of nanoparticles with distinct lifetimes. The lifetimes of these nanoparticles were well separated and independent of both the emission colour and intensity, making the nanoparticles particularly useful as optical encoding labels for multiplexed analysis. It is not immediately clear, however, how well the manipulation of luminescence lifetime through photon upconversion benefits multiplexed analysis after background subtraction. Additional experiments are needed to investigate the details of

energy transfer and dissipation that form the basis for tuning the time domains in these materials.

The availability of diverse time-domain codes also allowed Lu and colleagues to develop security inks with added technological complexity that rivals, or even exceeds, that of conventional printing inks. For example, they prepared solutions containing nanoparticles with three different emission lifetimes as printing inks, and used them to generate overlaid patterns with three layers. Recognition of the distinct layers in these patterns was readily achieved by time-resolved near-infrared light scanning (Fig. 1b). The ability to resolve superimposed two-dimensional images using lifetime-encoded nanoparticles could also enable the construction of new optical data storage systems with high densities and fast data readout rates.

Overall, the data and interpretation presented by Lu *et al.* not only provide fundamental insights into controlling

energy migration through lanthanide ions in NaYF<sub>4</sub> nanoparticles, they also, more broadly, highlight the critical role that lanthanide doping can play in tuning the temporal characteristics of upconversion nanocrystals. Coupled with recent advances in fluorescence lifetime imaging microscopy<sup>9</sup>, the experimental capabilities presented here may provide optical multiplexing solutions for living specimens with extremely high spatial and temporal resolutions. For biological imaging in aqueous solutions, a future issue that needs to be addressed is the risk of overheating associated with using 980-nm excitation. One possible way to overcome this problem is to use neodymium-sensitized

upconversion nanoparticles, which are attracting increasing interest<sup>10–12</sup>.

*Renren Deng and Xiaogang Liu are at the Department of Chemistry, National University of Singapore, Singapore 117543, Singapore and the Institute of Materials Research and Engineering, Singapore 117602, Singapore.  
e-mail: chmlx@nus.edu.sg*

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#### Correction

The print version of the Interview entitled “Warming up” (*Nature Photon.* **7**, 1008; 2013) contained an incorrect version of the photograph, which was missing one group member listed in the figure caption. This photograph has been replaced with the correct one in both the HTML and PDF versions of the Interview.