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Photoactivation of Luminescent Centers in Single SiO_2 Nanoparticles

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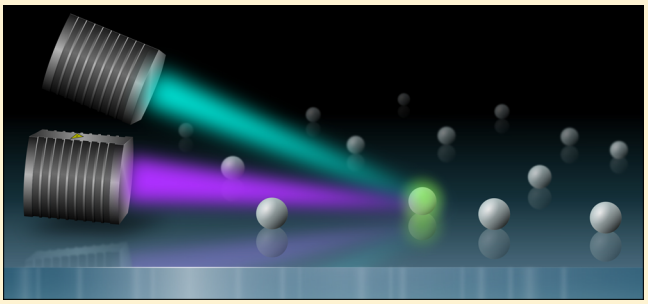
Supporting Information

ABSTRACT: Photobleaching of fluorophores is one of the key problems in fluorescence microscopy. Overcoming the limitation of the maximum number of photons, which can be detected from a single emitter, would allow one to enhance the signal-to-noise ratio and thus the temporal and spatial resolution in fluorescence imaging. It would be a breakthrough for many applications of fluorescence spectroscopy, which are unachievable up to now. So far, the only approach for diminishing the effect of photobleaching has been to enhance the photostability of an emitter. Here, we present a fundamentally new solution for increasing the number of photons emitted by a fluorophore. We show that, by exposing a single SiO_2 nanoparticle to UV illumination, one can create new luminescent centers within this particle. By analogy with nanodiamonds, SiO_2 nanoparticles can possess luminescent defects in their regular SiO_2 structure. However, due to the much weaker chemical bonds, it is possible to generate new defects in SiO_2 nanostructures using UV light. This allows for the reactivation of the nanoparticle's fluorescence after its photobleaching.



KEYWORDS: *Luminescent centers, nano-optics, fluorescence activation, fluorescence microscopy, photobleaching, single molecule spectroscopy*

Nanostructured silicon dioxide (SiO_2) has been the object of extensive studies since several decades. Due to its unique physicochemical properties,[1] nanostructured SiO_2 has found numerous applications in microelectronics,[2] targeted drug delivery,[3] cancer therapy,[4] synthesis of silica-encapsulated metal nanoshells,[5,6] and the fabrication of biological labels.[7,8] However, only little attention has been paid to the fluorescence of inherent defects in SiO_2 because of the high complexity of their photophysical properties.[9−12] A number of recent single-particle studies have shown that luminescent centers in individual SiO_2 nanoparticles possess a unique combination of emission properties, which have never been observed before. In particular, it has been shown that a particle can spontaneously switch its fluorescence from one luminescent center to the other, which leads to a sudden reorientation of the particle's transition dipole moment.[9] Godefroo et al. have shown that the luminescent defects in an ensemble of Si/SiO_2 core−shell nanoparticles can be created by exposing them to UV illumination.[13] Recently, it has been shown that luminescent defects in SiO_2 nanoparticles can undergo irradiation-induced conversion toward stable and metastable configurations possibly due to reactions at the surface sites with atomic or molecular species of the atmosphere.[14]

Here, we show the activation of luminescent centers in a single SiO_2 nanoparticle by illuminating it with UV light after its photobleaching. By analogy with nanodiamonds where the luminescent centers can be created by exposing a particle to a high-energy source,[15] creation of luminescent defects in SiO_2 nanostructure occurs upon breaking a chemical bond.[13] However, because of sufficiently weaker chemical bonds, the generation of defects in SiO_2 requires much lower energies, which allows for a reactivation of the nanoparticle's fluorescence within the same sample. This opens new perspectives for a drastic enhancement of the number of photons emitted by a particle. Moreover, a new approach to the photoinduced transition between the on- and off-states may potentially become a new tool for enhancing the spatial resolution in imaging by exploiting the stochastic photo-switching-based super-resolution microscopy techniques[16,17] or RESOLFT.[18]

We investigated the activation of luminescent centers in single SiO_2 nanoparticles of several diameters from 11 to 166 nm, which had been synthesized using a modified Stöber method in a biphasic system using an amino acid as a base catalyst.[19,20] A recent comprehensive fluorescence study of 11 nm sized SiO_2 nanoparticles,[12] which were prepared using the same method, has shown that the photophysical properties of the luminescent centers in these SiO_2 nanoparticles partly

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resemble those of typical single dye molecules. For instance, it was observed that the fluorescence from these particles exhibits a linear transition dipole, and symmetric emission and excitation spectra. However, a strongly inhomogeneous local chemical environment around the centers results in a broad and random variation of the single nanoparticle emission and excitation spectra, fluorescence lifetime, and quantum yield. In particular, both the emission and excitation spectra of different luminescent centers can exhibit a shift within the range 2.0−2.4 eV. It is remarkable that, despite a broad variation of the shift of both the emission and excitation single-particle spectra, the shape of the spectrum remains constant, which suggests that the fluorescence stems from the same type of defect.

Despite the large number of investigations that try to understand the fundamental mechanisms of the fluorescence originating from different types of defects and their localization in SiO_2 nanostructures, many details are still unclear. In particular, the ensemble fluorescence of identical SiO_2 nanoparticles (2.0−2.4 eV)[12] spectrally partly overlaps with those of the several possible types of luminescent centers in SiO_2: neutral oxygen vacancy center (≡Si−Si≡),[21−24] isolated nonbridging oxygen atom (≡Si−O$^{\bullet}$),[22,25,26] hydrogen-related groups (≡Si−H and ≡Si−OH),[27] silanone surface groups ((≡Si−O)$_2$Si═O),[28] a pair of a dioxasilirane, (═Si(O$_2$)),[14] and a silylene (═Si$^{\bullet\bullet}$).[14] Whereas the spectral information makes it difficult to attribute the observed emission to the particular type of luminescent center, we can obtain information about the localization of defects within the particle. We performed fluorescence quenching experiments on SiO_2 nanoparticles in aqueous solution using sodium iodide. Particles of all sizes exhibited a strong fluorescence quenching comparable to the one observed for rhodamine dye molecules (Figure S3). The high accessibility of the luminescent centers to the quencher suggests that the centers are located on the surface of the particle. As all of the above types of defects can be located on the surface of the particles, an unambiguous attribution of the observed fluorescence requires further investigation. We will postpone the discussion of the specific types of luminescent centers to the end of this paper.

For the single-particle photoactivation studies, SiO_2 nanoparticles were deposited on the surface of a clean glass cover slide by spin-coating a 20 μL droplet of low concentrated aqueous solution. All of the glass cover slides used in this study were cleaned according to a procedure described in the Supporting Information. The cleaned substrates were verified to have very low fluorescent contaminations, which did not influence the results of the study. Deposition of the particles on the substrate surface without use of polymer matrix for their immobilization guarantees that all of the particles are in identical chemical environment.

Figure 1a illustrates the scheme of the custom built confocal microscope. Excitation was done with a pulsed laser beam at 488 nm, with a total power of 10 μW, and a pulse repetition rate of 20 MHz. The excitation pathway was equipped with a polarization converter, which allowed us to scan individual particles with an either azimuthally or radially polarized laser focus. This allows one to discern the excitation dipole orientation of a luminescent center. Observation of a single transition dipole excitation pattern for both an azimuthal and radial beam excitation suggests that the observed fluorescence stems from a single fluorophore (Figure 1c and d). The second excitation line allowed us to focus simultaneously a Gaussian laser beam (488 nm, 10 μW, 20 MHz repetition rate) for

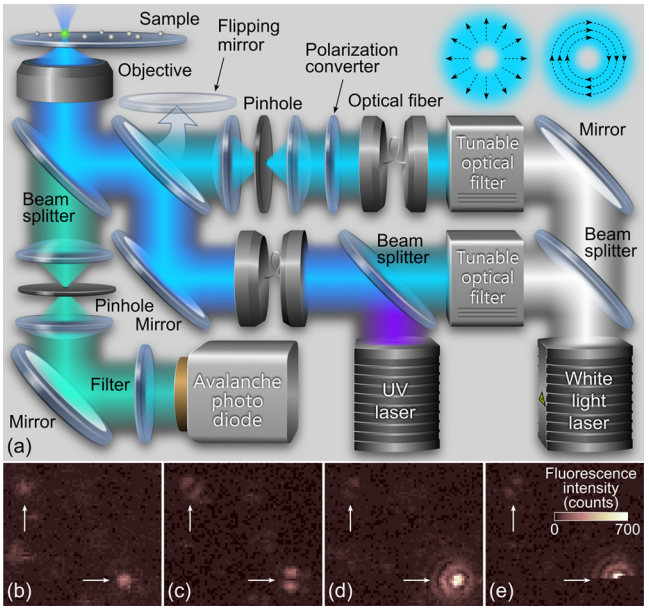

Figure 1. (a) Scheme of the experimental setup. Right upper corner: cross sections of a radially (left) and azimuthally (right) polarized laser beam, generated by the polarization converter optical line. Confocal scanning images of the same sample area recorded using a Gaussian (b), azimuthal (c), and radial (d) and (e) beams. The white arrows show the location of two luminescent nanoparticles. Image (e) shows a photobleaching event of the lower right particle.

excitation of the particles, and UV light (378 nm, 200 μW, 40 MHz repetition rate) for activation of luminescence centers within the same focal area by coupling both beams into the same optical fiber. The two excitation wavelengths have been selected so that illumination of a particle with UV light for activation of fluorescence does not contribute to excitation of the particle's fluorescence in the measured spectral range. According to the width of a single SiO_2 nanoparticle excitation spectrum measured in ref 12, separation of the wavelengths for activation and excitation of fluorescence by 110 nm allowed us to separate the processes of the photogeneration of luminescent centers and their excitation.

At first we would like to discuss the results of the 11 nm particle photoreactivation. Figure 2a shows a fluorescence time trace recorded upon excitation of a single SiO_2 nanoparticle with a focused 488 nm Gaussian laser beam. It exhibits a single on-state and a single-step transition to the off-state, which indicates that fluorescence stems from one individual quantum emitter. Further excitation of the particle with 488 nm excitation light did not lead to the detection of fluorescence.

For reactivation of the single particle photoemission, the particle was exposed to both 488 and 378 nm focused excitation light. The measured time trace (Figure 2c) exhibits two on-states, separated by time gaps during which the particle was in the off-state. The single step transitions between the on- and off-states suggest that all the observed fluorescence originates from one single luminescent center. To determine whether the observed emission on-states are related to excitation of the same center or to the activation of a different one, we determined the excited state lifetime values for each of the observed on-states. It has been shown, that due to the different local chemical environment around luminescent centers in SiO_2 nanoparticles, fluorescence lifetime values can vary by a factor of 10 for different centers of the same type.[12]

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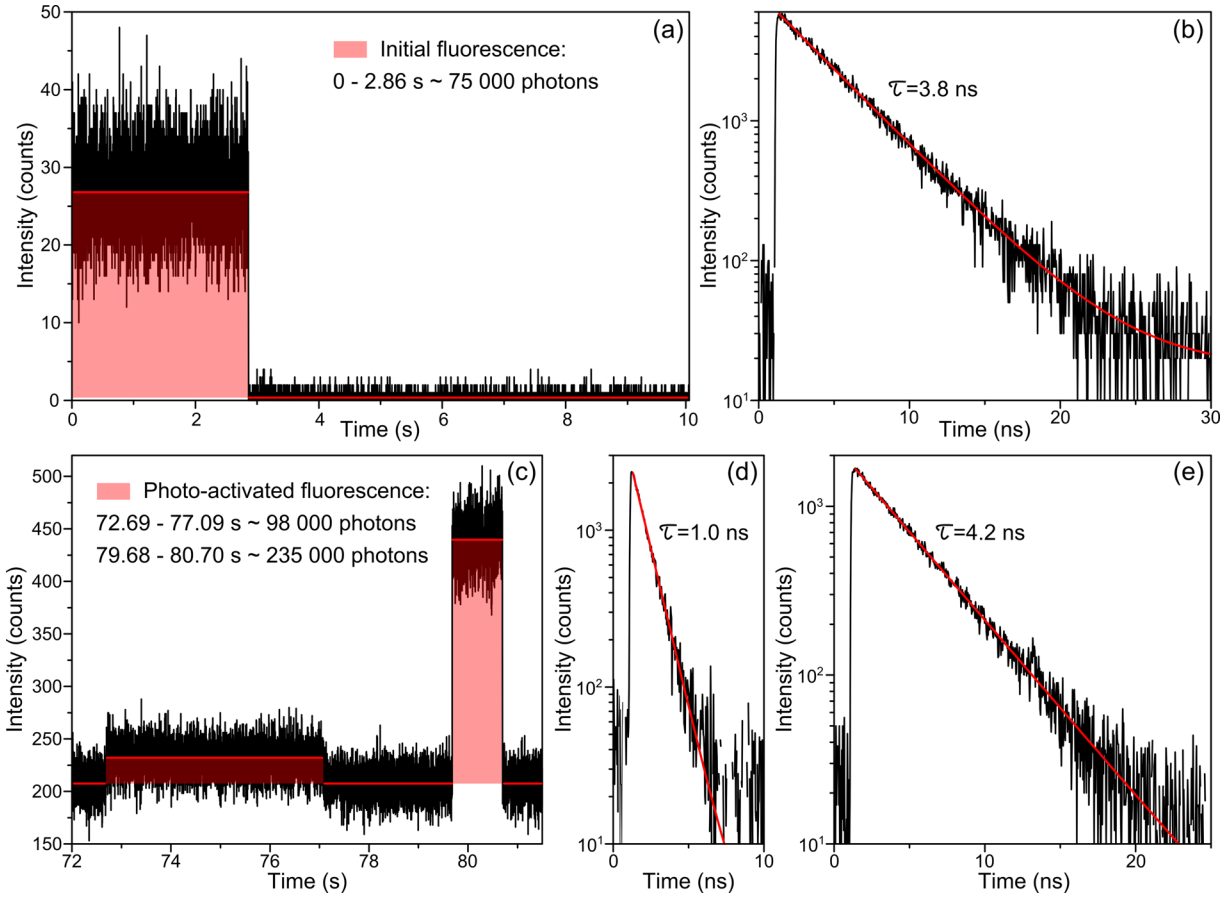

Figure 2. Fluorescence time traces of initial (a) and photoactivated (c) fluorescence. The red horizontal lines show the average signal intensity level for the on- and off-states of the fluorescence. The red shaded areas indicate the total number of photons detected from the particle. Histograms of the photon arrival times for the initial (b) and photoactivated fluorescence (d) (72.69−77.09 s) and (e) (79.68−80.70 s). The red curves represent the fit to the measured data with a monoexponential function.

Therefore, the excited state lifetime is an individual signature of a luminescent center in a SiO_2 structure.

For determining the fluorescence lifetime values corresponding to different on-states, we plotted a histogram of the arrival times of the detected photons separately for each of the on-states. All of the obtained fluorescence decay curves both for the initial and reactivated fluorescence (Figures 2b, d, and e) could be well fitted with a monoexponential decay function, which is an additional indication that the fluorescence originates from single excited energy states, that is, from single luminescent centers. The fluorescence lifetime values for different on-states showed a sufficient difference suggesting that the observed on-states are related to photoactivation of different luminescent centers. This finding is in agreement with the previous observation of rare spontaneous reorientations of the particles' excitation transition dipole moment,[9] which indicates switching of the fluorescence between different emitters. We detected near 10^5 photons per luminescent center, which corresponds to the photostability of some widely used dye molecules[29] and exceeds those of most fluorescent proteins.[30] Note that the particles are not embedded into a solid matrix and therefore are easily accessible to atmospheric oxygen, which potentially reduces their photostability.

In total, we observed photoreactivation of fluorescence from 16 of 200 nanoparticles of 11 nm in diameter. Figures S4−S6 show more examples of fluorescence time traces measured from individual SiO_2 nanoparticles, which exhibited emission reactivation after photobleaching. Analysis of the fluorescence on-state duration and the time point of fluorescence photoactivation for all of the 11 nm sized nanoparticles, which exhibited photoactivation of fluorescence (Figure S7), revealed that both parameters are distributed within a relatively broad range and show no clear correlation among the measured particles. However, it is remarkable that most of the photoactivation events occurred within the first 1.5 min after turning UV radiation on. To make sure that the fluorescence photoreactivation statistics is not influenced by contaminations, we performed identical experiments on fluorescence activation on the surface of a clean glass cover slide without particles. We did not observe any fluorescence events for all of the 200 randomly selected points.

Now, we will discuss the dependence of the fluorescence photoactivation efficiency on SiO_2 nanoparticle size. We performed identical experiments on the photoactivation on 200 initially luminescent particles of each of the diameters (11, 29, 50, 98, 122, and 166 nm). Figure 3 shows the number of particles which exhibited reactivation of emission versus particle diameter. The drastic decrease of photoactivation efficiency for the chemically identical nanoparticles raises the question of its origin. As the excitation intensity plays one of the key roles in fluorescence reactivation, we calculated the intensity distribution for the 378 nm excitation around the particles of different sizes. Figure 4 shows the field intensity distribution in case of the presence (a−f) and absence (g−l) of particles. For better

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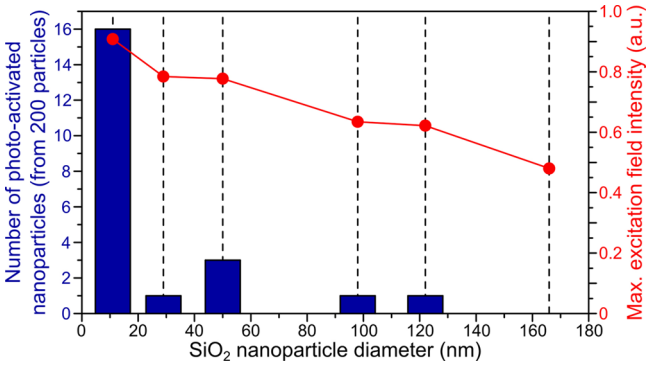

Figure 3. Histogram: Number of reactivated SiO_2 nanoparticles from the total 200 measured particles versus nanoparticle diameter. Red solid circles: Maximum excitation field intensity near the surface of SiO_2 nanoparticles of different sizes (see Figure 4 for more details). The dashed lines indicate the sizes of the particles studied.

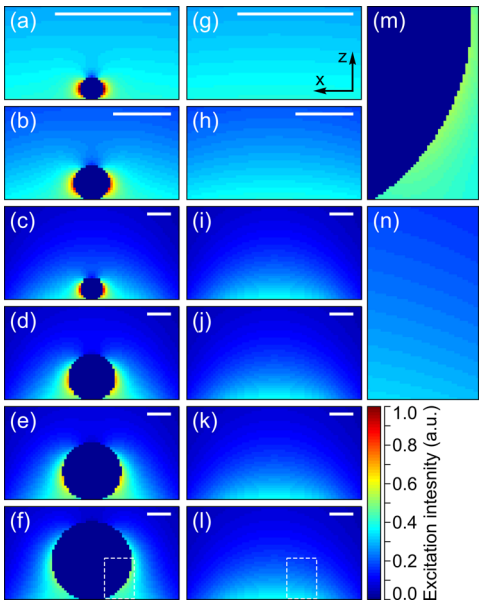

Figure 4. Calculated distributions of the excitation field intensity. The calculation is done for 378 nm linearly polarized laser beam focused with 1.49 numerical aperture objective lens at the glass-air interface. (a–f) The SiO_2 nanoparticles of different sizes are placed on the glass surface in the center of the focal spot. (g–l) Calculations in absence of the nanoparticles. Scale bars 50 nm. (m and n) Excitation field intensity distribution within the area shown with dashed curves in images f and l, respectively, calculated with the same spatial resolution as the one in image (a). All of the images are normalized to the same intensity scale.

comparison, all figures are shown with the same intensity scale. It is remarkable that the presence of a SiO_2 nanoparticle in the center of the focus leads to an enhancement of the excitation field around the particle. Moreover, the field enhancement grows with decreasing particle size and reaches a factor of 2.1 for 11 nm particles. As the luminescent centers are found to be located at the surface of the nanoparticles, the change of maximum field intensity near the particle's surface for different particle sizes can strongly modify the photoactivation efficiency.

To make sure that the change of the excitation field intensity maximum for different particle sizes is not an artifact of the spatial resolution of the calculated images, we computed the indicated subarea of Figure 4f (dashed rectangle) with the same resolution as the one of image (a). Figure 4m shows that the excitation field intensity maximum around the 166 nm sized particle is nearly 2 times lower than for nanoparticles of 11 nm diameter.

Solid circles in Figure 3 show the modulation of the excitation field intensity maximum at the particle's surface for SiO_2 nanoparticles of different sizes (placed in the center of the 378 nm focused laser spot). Despite the change of the field intensity maximum, the obtained dependence cannot fully explain the steeper modulation of the fluorescence activation efficiency.

We assume that another key factor, which determines the dependence of photoactivation efficiency on particle size, is distortion of chemical bonds on the surface of ultrasmall nanoparticles. It has been shown that reducing the SiO_2 particle size from 400 to 7 nm changes the Si−O−Si bond angle from ∼180° to ∼165°.[31] As a result, the presence of surface strains increases chemical reactivity and can induce the formation of structural defects.

By analogy with the emission centers in nanodiamonds,[32] trapping of a charge carrier in close proximity to the luminescent center in a SiO_2 nanoparticle may also lead to a modification of its luminescence properties. However, the almost full absence of blinking suggests that the relation of the observed photoswitching to the charge trapping is unlikely.

According to the model proposed by Glinka et al.[27] and later further investigated by Rahman et al.,[31] the size-dependent photoactivation efficiency speaks in favor of attribution of the observed green fluorescence to the hydrogen related species. This assumption is also confirmed by the strong spectral overlap of the ensemble signal with maximum at near 2.27 eV observed in the current study[12] with fluorescence at 1.8–2.8 eV reported in the above works. However, this model contradicts the growth of the defect-related fluorescence in Si/SiO_2 core–shell nanoparticles after dehydrogenation of the sample and exposure to UV radiation.[13]

The high complexity of the photophysical and physicochemical properties of SiO_2 nanoparticles, which have been observed in the current study and in previous works, requires their further investigation. We believe that further progress in understanding the structural and optical properties of SiO_2 nanostructure can be achieved in comprehensive studies, which combine fluorescence microscopy and X-ray photoelectron spectroscopy and/or transmission electron microscopy. Moreover, modeling of SiO_2 nanoparticles structure may provide a new insight into the distortion of chemical bonds on the surface of ultrasmall nanoparticles. Understanding the generation mechanism of radiative and nonradiative defects in SiO_2 structure would open new possibilities to control emission of photons from silicon nanocrystals, where fluorescence can stem from localized emission centers in the SiO_2 shell.[33,34]

In the current work, we have shown the new effect of the photoactivation of luminescent centers in SiO_2 nanostructures at the single particle level. We envision a great potential of this effect for various applications of luminescence spectroscopy and microscopy, which currently suffer from the limited photostability of fluorophores. The next important step will be to systematically investigate how to dramatically increase the efficiency of luminescence activation in SiO_2 nanostructure. We assume that the key factor of increasing the activation efficiency is adjusting the power and wavelength of the activating radiation to the specific properties of particular types of luminescent centers in SiO_2. Whereas this can be achieved by

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modulating the parameters of the light source, another idea would be to couple SiO_2 nanoparticles to plasmonic nanostructures,[35] which would allow one to enormously enhance the activation field around the nanoparticles. This would greatly relax the necessity for high intensity and tightly focused UV light and would allow one to tailor the activation field parameters far below the diffraction limit. The observed activation yield for the smallest 11 nm sized nanoparticles, together with the biocompatibility of both SiO_2[5,7,8] and metal[36−38] nanoparticles, suggests the possibility of using fluorescence photoactivation for in vivo bioimaging. Finally, single particle photoactivation can potentially become a powerful tool for photoswitching-based super-resolution microscopy techniques, such as STORM, SOFI, or PALM,[16,17] providing with a new way for tailoring fluorescence on−off transitions. Moreover, a random shift of single particle fluorescence spectra and lifetimes, which are individual for each luminescent center,[9,12] can be used as additional parameters for enhancing resolution. However, accurate tailoring of the photophysical properties of luminescent centers in SiO_2 nanoparticles and increasing their photoactivation efficiency requires further research of their complex properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.6b01361.

More detailed information regarding the synthesis of silica nanoparticles and size distribution analysis, generation of azimuthally and radially polarized laser beams, experimental methods, and the supplementary figures (PDF)

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Notes

The authors declare no competing financial interest.

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