Selective placement of quantum dots on nanoscale areas of metal-free substrates

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We demonstrate a novel approach for selectively immobilizing semiconductor core–shell quantum dots directly on metal-free substrates with nanoscale resolution. This is accomplished by defining a mask via electron-beam lithography (EBL) followed by the functionalization of only the exposed areas of the substrate with quantum dots using a heterobifunctional linker. Non-specific binding is suppressed by a binding inhibitor.

1 Introduction The study of hybrid photonic systems consisting of quantum emitters coupled to sub-wavelength nanophotonic structures, including nanoantennas and nanoscale resonators, remains a vibrant field of research [1]. In particular, a huge effort is concentrated on experiments that employ colloidal semiconductor quantum dots (QDs) as emitters, owing to their high quantum yields, photo-stability, and chemical stability, as well as to the large range of available wavelengths [2–7]. In such coupled QD-resonator systems, a range of interesting effects can be observed, including strong Purcell enhancement [3], directional emission [4, 5], polarization-engineered emission [5], excitation enhancement [6], and broadband emission enhancement [7]. For the mentioned effects the placement of QDs at specific locations allows for enhanced functionality, performance, and control of emission properties. However, with a few exceptions [4, 6], most of these earlier works do not provide control over the lateral positioning of the QDs with respect to the photonic nanostructures. This is mainly due to the fact that the precise and controlled placement of colloidal QDs with respect to arbitrary nanophotonic structures by a scalable lithographic process still poses a major challenge. Recent breakthroughs have been discussed in Ref. [1] and include selective binding of QDs to the surface of gold structures [4, 6, 8], as well as creating closely packed aggregations of PbS QDs on a quartz substrate [1] and patterning of nanocrystal films on silicon oxide substrates [9]. A number of other methods for the selective placement of QDs based on various lithographic techniques has also been presented before [10–15]. However, none of these approaches has been shown to allow for the assembly of small ensembles of QDs on a glass substrate by a scalable lithographic process with nanoscale resolution. In particular, methods that rely on the formation of closely packed QD ensembles [1, 9] have a number of drawbacks regarding their use for applications in nanophotonics. Firstly, these methods conceptually do not allow for placement of individual QDs. Secondly, close packing of QDs can lead to QD photoluminescence (PL) quenching. Third, these methods usually employ a lift-off procedure after QD deposition, resulting in harsh treatment of the QDs with a potentially
negative impact on their optical properties. On the other hand, single QD placement has been demonstrated for methods that employ specific binding of QDs to a functionalized metal surface [4, 6, 8]. Indeed optical spectra as well as blinking traces have been presented for specific binding to metal nanostructures [4]. Here, the challenge lies in the generalization of these methods that would enable the precise placement of QDs on a metal-free substrate. This development is hampered by strong non-specific binding of the QDs to the glass surface, which hinders position-selective immobilization of QDs. A new demand for a procedure for selective placement of QDs to metal-free surfaces arises from the recent developments in the fields of all-dielectric nanoantennas [16, 17] and all-dielectric metamaterials [18], which are currently receiving lots of attention because of their low intrinsic losses, and strong magnetic response. In particular, coupling of QDs to all-dielectric nanoresonators is expected to generate highly directional emission patterns [16, 17].

2 Selective QD immobilization

Here, we demonstrate an electron-beam lithography (EBL) based process to selectively bind colloidal semiconductor core–shell QDs to pre-defined regions of a transparent, metal-free substrate. We choose indium-tin-oxide (ITO) coated glass as the substrate material, as it combines the advantages of being electrically conductive for EBL and transparent for optical transmittance measure-ments, resulting in its widespread use in subwavelength nano-optics. Importantly, the QDs used in our work are well isolated from the conductive ITO layer by their shell and additional polymer and biomolecule coatings, and we can observe bright emission from these QDs on the employed substrates. Note also that the ITO layer thickness is kept below the percolation threshold, such that the uncovered parts of the underlying glass substrate are still available for surface chemical reactions. We have checked with initial experiments, which did not involve EBL, that the actual surface functionalization procedure employed in the following also works for silicon samples as well as for plain glass substrates without ITO coating. The individual steps of our QD placement process are illustrated in Fig. 1. First, standard microscope glass cover slides are sputter coated with 7 nm ITO to render them conductive for EBL. The substrates are then spin-coated with the positive-tone electron-beam resist PMMA A4 (Microchem, 6000 rpm, 90 s). We prepare a PMMA mask by EBL, exposing the substrate surface only at the positions intended for QD immobilization, namely squares with a footprint of 3 μm × 3 μm arranged in a two-dimensional array with 10 μm center-to-center distance. Next, we apply an oxygen plasma for 10 s, leading to functional hydroxyl (OH) groups on the substrate surface. We dissolve 1.5 mg of the heterobifunctional linker, Silane-PEG-Biotin MW 1000 (Nanocs Life Research), in 1.5 ml 95% ethanol. We then apply the linker solution to the sample surface and let it react for 10 minutes at 40 °C. This allows the silane groups of the linker molecules to attach covalently to the exposed areas of the substrate. We wash the sample with isopropyl alcohol to remove any unreacted linker, as well as to remove water residue that can hydrolyze the silyl bonds. We then perform a standard PMMA lift-off procedure by inserting the sample into hot acetone at 80 °C for several minutes. Next, in order to inhibit non-specific binding of streptavidin coated QDs to the sample surface outside of the functionalized areas, we apply a solution of 0.5% vol/vol horse serum (Life Technologies) in incubation buffer solution (Qdot® incubation buffer, Life Technologies), which is routinely used in Life Sciences as blocking agent [19]. We leave it to adsorb for approx. 1 h, and remove the excess by washing the sample with incubation buffer solution. Finally, we apply a solution of streptavidin-coated CdSeTe/ZnS core-shell QDs (Qdot® 800 IT™ streptavidin conjugate, Life Technologies), diluted 1:100 in incubation buffer solution, allowing the streptavidin to bind to the biotin groups of the substrate-bound linker molecules. After 10 minutes, we wash the sample with incubation buffer solution and blow-dry with air. It is worth noting that applying the QD solution as a last step in the process after the mask lift-off, has several advantages: Most importantly, the QDs are not exposed to any harsh treatment, preventing a negative impact on their optical properties. Furthermore, the absence of notable sample topography, i.e., the fact that the sample is flat at the time of application of the QD solution, rules out any systematic topographically induced aggregation effects of the QDs. This enables good control over the finally resulting area-density of deposited QDs inside the functionalized areas, and in principle allows for depositing on average a single QD inside a given area for an optimized concentration of the QD solution.

3 Optical characterization

The samples are optically characterized with an Olympus inverted microscope...
clusters of QDs can be distinguished in this image, which limited bright spots corresponding to individual or small by the white dashed box in Fig. 2(a). Individual diffraction PL image. Figure 2(b) shows a close-up of the area marked have been exposed to the linker solution appear brighter in the field of view, across a spectral range of 405–807 nm. A PL overview images are collected using a 40× objective. Close-up images are obtained using a 100× oil immersion objective. An additional 1.6× magnifying lens in the imaging port allows for resolving single QDs, which appear as individual diffraction-limited bright spots. A hyperspectral imaging system (HSi-300, Gooch and Housego) was inserted in the camera path to acquire the PL emission spectra of the imaging area, and was controlled using the open source microscopy software Micro-Manager [20]. The system allowed simultaneous acquisition of PL emission spectra of the whole camera field of view, across a spectral range of 405–807 nm. A PL intensity overview image of the fabricated sample is shown in Fig. 2(a). Clearly, the regions where the substrate has been exposed to the linker solution appear brighter in the PL image. Figure 2(b) shows a close-up of the area marked by the white dashed box in Fig. 2(a). Individual diffraction limited bright spots corresponding to individual or small clusters of QDs can be distinguished in this image, which have a much higher density inside the exposed areas of the substrate than outside. In order to unambiguously attribute the observed bright areas to QD PL we record the PL emission spectrum of the bright area in Fig. 2(a). These results are shown in Fig. 2(c), confirming that the PL has a spectral maximum around a wavelength of 790 nm. This is in good overall agreement with the specified QD emission spectrum (grey curve) apart from a slight blue shift of about 10 nm. The QD PL peak is superimposed onto a broad PL background signal (red curve), which is spectrally, spatially, and temporally constant within the noise level, and which we attribute to the fluorescence of the substrate as well as dark-noise and read-out noise associated with the camera.

4 Observation of QD blinking So far we have confirmed that our process can indeed strongly influence the arrangement of the QDs on the substrate. However, significant numbers of stray QDs outside of the exposed areas as well as spillover effects are still apparent. This leads to a blurred appearance of the QD functionalized patterns, effectively limiting the precision of the placement process. In order to address these issues, we repeat the process with a refined PMMA mask featuring patches with footprints reaching dimensions below 100 nm × 100 nm. A scanning electron micrograph of this mask is presented in Fig. 3(a). Furthermore we reduce the concentration of QDs in the solution by a factor of 10. The resulting PL image for the improved process is displayed in Fig. 3(b). Clearly, the positions of the bright spots in Fig. 3(b) coincide very well with the layout of the PMMA mask, with minimal non-specific binding. The brightness of the individual spots varies depending on the number of QDs that are positioned within the corresponding site. By monitoring the PL images in real time we observe that most of the bright spots situated at the sites defined by the PMMA...
mask exhibit pronounced brighter-fainter or on-off cycles caused by blinking of the QDs. While blinking is often not desirable, in particular if QDs are used as biological markers, the observation of a series of defined on and off states in QD PL time traces is indicative of emission from a single QD [4, 21]. As such, although more complex methods like, e.g., antibunching experiments or high-resolution atomic-force microscopy are necessary to provide unambiguous evidence for single QD placement, QDs that do show blinking are convenient probes for providing a first indication of the placement of singles or small clusters of optically functional QDs. In order to provide evidence of this blinking we record time traces of PL intensity at various nominal functionalized-patch positions. In Fig. 3(c)-(h) we present the PL and blinking characteristics for two example areas marked by the small white squares denoted #1 and #2. To obtain the time traces depicted in Fig. 3(e), (f) we average the per-pixel PL signal over the 7 × 7 pixel areas marked by the white boxes in Fig. 3(c), (d), respectively, and subtract the background signal obtained from a QD free region of equal size. The time interval is 0.05 s. QD blinking is clearly observed for both sites, and further confirmed by the two-peak characteristics of the time-trace histograms displayed in Fig. 3(g), (h). In order to provide statistical information about the success rate of our process under the employed experimental conditions we furthermore analyse the time traces for an entire row of 30 nominal functionalized-patch positions. We find clear 2–4 peak characteristics indicative of single/double QDs at the respective site for 16.7% of the analyzed fields. Furthermore, for 86.7% of the nominal functionalized-patch positions we find defined bright spots, indicating that at least one QD has been assembled at these sites. These success rates could be further enhanced by using more/less diluted QD solution, respectively.

5 Conclusion In conclusion, we have demonstrated a fast and easy EBL-based process to selectively bind colloidal semiconductor core-shell QDs to ITO coated glass substrates with submicron resolution. This process does not rely on the presence of metals. It involves only commercially available substances and makes use of the reliable biotin-streptavidin binding mechanism widely employed in biophotonics [22], while inhibiting non-specific binding with a blocking agent. Our approach allows for placement of small ensembles of nanoparticles, and the particle density can be influenced by tuning the size of the functionalized areas and the concentration of the QD solution. Precise positioning of QDs with respect to an arbitrary photonic nanostructure can be achieved by joining the two-step EBL process with an alignment procedure. While the employed QDs are available for a large range of visible and near-infrared emission wavelengths, the demonstrated process can in principle also be generalized for the assembly of any other kind of streptavidin-coated nanoparticles, like upconversion nanocrystals or nano-diamonds.

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References