Supporting Information for

Quantum dot triexciton imaging with three-dimensional subdiffraction resolution

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EXPERIMENTAL SECTION

Quantum dots and cellular labeling

QDot655 quantum dot streptavidin conjugates (Q10121MP) and Qdot655 goat F(ab')2 anti-mouse IgG conjugates (Q11021MP) were purchased from Invitrogen, USA. As primary antibodies for immunofluorescence of microtubules in COS-7 cells, monoclonal anti-β-tubulin (T5201, Sigma) were used. Surface preparation was performed spin coating 50 to 75 µl Qdot655 dissolved in water at a concentration of 1 nM onto a glass cover slide. Cover slides were treated with hydrofluoric acid (3%; Sigma, USA) prior to use. For three-dimensional confocal scanning of single QDs, a 1 nM QDot655 solution in 3% polyvinyl alcohol (PVA; Sigma) was prepared and spin coated onto the cover slides. For fixed cell staining African green monkey kidney COS-7 cells were plated in LabTek 8-well chambered coverglass (Nunc). After 12 to 24 hours, the cells were fixed with methanol for 10 min. The fixed cells were washed with PBS, permeabilized (PBS containing 0.5% v/v Triton X-100) for 10 min, and treated with blocking buffer (PBS containing 5% w/v normal goat serum for 30 min). Microtubules were stained with mouse monoclonal anti-ß-tubulin antibodies for 60 min. Afterwards the cells were stained with Qdot655 labeled goat anti-mouse F(ab')2 fragments overnight. After each staining step three washing steps using PBS containing 0.1% v/v Tween 20 were applied. For internalization of QDs into living cells COS-7 cells were plated in LabTek 8-well chambered cover glass. Living cells were incubated in presence of 10-100 nM QDot655 quantum dot streptavidin conjugates for 2-4 hours in a 5% CO2 incubator at 37°C. After incubation the cells were washed gently for multiple times and kept in Leibovitz's L-15 Medium (Invitrogen).

Optical setups

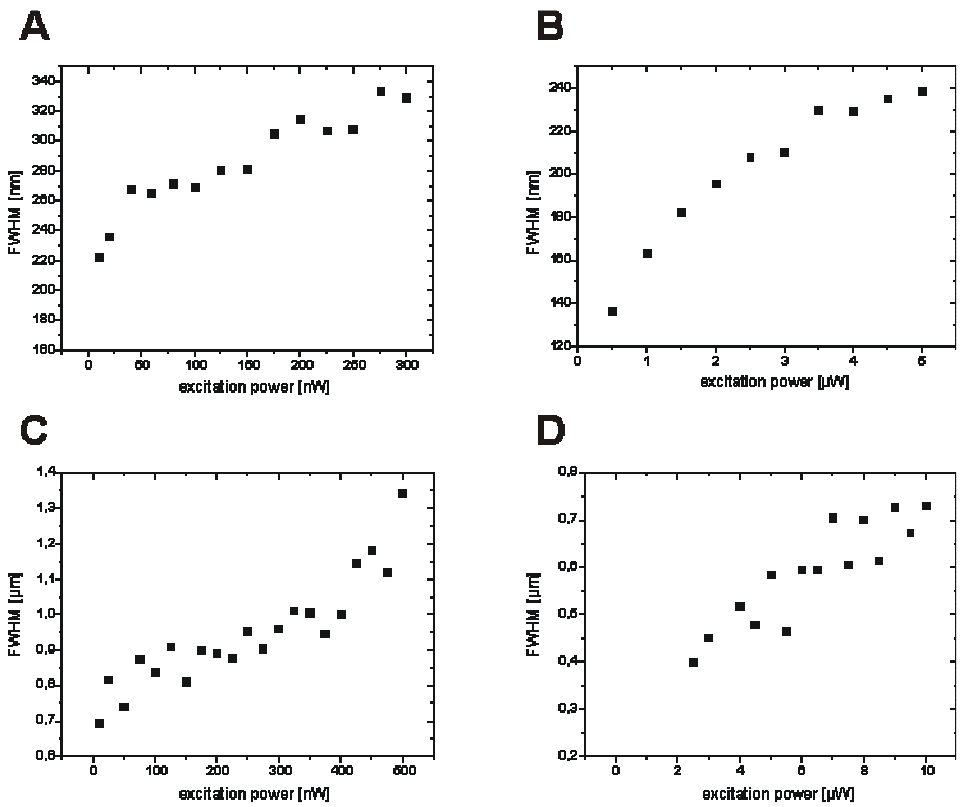
All measurements were performed at room temperature (25 °C). A standard confocal setup (Fig. 1a) was used for the experiments and was essentially described elsewhere (1). For the excitation of Qdot655 quantum dots, a 445-nm pulsed diode laser with a pulse length of ~ 100 ps (Picoquant GmbH, Berlin, Germany) was coupled into a single mode fiber and operated with a repetition rate of 5 MHz. The laser light was directed into a 100x oil immersion objective (Zeiss Apochromat, NA = 1.45; Zeiss, Jena, Germany) passing a dichroic beamsplitter (DHCP465; AHF Analysentechnik, Tübingen, Germany). The fluorescence light was collected by the same objective and separated from excitation light by a long pass filter (500LP, AHF). The fluorescence light was spectrally separated into two channels and directed onto the active areas of two avalanche photodiodes (APD; Perkin Elmer, USA) by a dichroic beamsplitter (600DRLP) in the detection path. The fluorescence signal on the two detector

channels was further filtered with a 575DF50 and a 675DF50 bandpass filter. The signal of the detectors was processed by a PC-card for time-correlated single-photon counting (TCSPC 630; Becker&Hickl, Berlin, Germany). The sample was scanned with a scanning stage (Physikinstrumente, Göttingen, Germany) with a pixel size of 50 nm and an integration time of 1 ms / pixel. The acquisition time of a typical image of 200 x 200 pixel size was 40 seconds. Custom written LabView software (National Instruments, USA) was used for data acquisition. Data analysis was carried out with Origin8 (MicroCal, USA) and custom written LabView software.

Whole-cell three-dimensional (3D) fluorescence imaging and live cell imaging was performed on a commercial beam scanning confocal fluorescence microscope, LSM 710 (Zeiss). The microscope was equipped with a continuous-wave argon ion laser, providing the two main wavelengths 488 and 514 nm. Detection channels were set at 550 – 600 nm (triexciton emission channel) and 650 to 700 nm (monoexciton emission channel). A variable pixel size between 50 and 200 nm and imaging frame rates of 1 to 10 Hz were used, allowing for 3D imaging within seconds. Choosing an excitation wavelength of 488 nm might lead to an increase in background intensity in the triexciton channel due to Raman scattering of water (590 to 600 nm), which can be reduced by shifting the emission window (to 550 – 590 nm) or using a different excitation wavelength (458, 473 and 514 nm are commonly provided by continuous wave argon ion laser sources).

Statistical Analysis of the axial and lateral point-spread function

The point spread function of single quantum dots QDot655 adsorbed on bare glass surface or embedded in a polyvinyl matrix (for 3D analysis) was analyzed by determining the full width half maximum (FWHM) in both lateral and axial direction for the triexciton and the monoexciton channel (including biexciton emission). The FWHM was determined by approximation with a Gaussian function, and with respect to the laser excitation intensity. A statistical analysis of 10 individual quantum dots is shown in Supporting Information Fig. 1. From the FWHMs measured at low excitation intensity (136 nm for the triexciton and 220 nm for the monoexciton channel) a resolution enhancement of 1.6 was determined in lateral direction (Figs. 1A and 1B). Accordingly, the experimental resolution enhancement in axial direction was determined to 1.7 (Figs. 1C and 1D, using FWHMs of 410 nm for the triexciton and 690 nm for the monoexciton channel). The FWHM values were selected with respect to similar count rates detected for monoexciton and triexciton emission.



Supporting Figure 1. The FWHM of the point spread function was determined from 10 individual quantum dots by approximation with a Gaussian function, both in lateral (A, B) and in axial (C, D) direction for the monoexciton (A, C) and triexciton emission channel (B, D).

References

1 M. Heilemann *et al.*, *J Am Chem Soc* **126**, 6514 (Jun 2, 2004).