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Tracking the dynamics of single quantum dots: Beating the optical resolution twice

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Abstract

Using particle tracking routines the location of single point light sources can be determined with an accuracy of a few nanometers. By using quantum dots (QDs) emitting at different wavelengths, the measurement of the distance between these point light sources, which are closer than the optical resolution of 200 nm, was achieved. The nanocrystals have major advantages over conventional chromophores in higher quantum yield, more photostability, and the possibility of different emission wavelengths by an excitation with a single wavelength. The colocalization of two single QDs at the least distance of 40 nm can be measured with a standard deviation of 5 nm and a time resolution of 117 ms using one excitation wavelength.

Introduction

Recent advances in biosciences allow now the accumulation of an unprecedented amount of information about the expression of specific genes and the subsequent translation into proteins. A major challenge of future biological research is the study of the dynamics of the interactions of these cellular constituents, in model systems as well as in cellular systems. Although most commonly used techniques allow only static observations at distinct time points, the study of the dynamics of the interaction is essential for an deeper understanding of the relevant biological processes. The biomolecular interactions occur on a nanometer scale, well below the typical optical resolution, given by the Rayleigh criterium. Typically, the Rayleigh criterium limits the resolution of optical imaging systems to approximately half the wavelength of light, which is far too large to isolate the location and motion of single chromophores. The experimental techniques, which overcome this physical limitation, are FRET, NSOM and single particle tracking. The recent application of these techniques on a single molecule level provides us with an unprecedented understanding of biological processes such as enzyme kinetics, lipid diffusion in bilayers or DNA properties, see for example [1–3].

It is, however, feasible to locate the position of a single chromophore through the use of fluorescence. The diffraction pattern from a point source is well known to be symmetric; thus it is possible to locate the center position of a point source to within approximately 5 nm from the optical diffraction pattern [4]. Unfortunately, if there is more than one chromophore, however, their relative positions can no longer be determined as accurately since the resolution is limited to the Rayleigh criterion. This limit can be overcome through the use of several different chromophores, which separately label different molecules; then each color can be resolved separately within a few nanometer, where the resolution depends only on the signal-to-noise ratio (SNR). Thus, multicolor tagging allows the tracking the positions of chromophores which are closely located within a range of a few nanometers.

This concept has been applied using conventional fluorescence molecules [5,6]. However, the application has been limited by four main disadvantages of these

tags: First, conventional fluorophores with distinct emission spectra have to be excited with different wavelengths. Consequently, the excitation wavelengths have to be switched during the measurement or well-adapted filter sets have to be used for simultaneous excitation. In both cases chromatic aberrations have to be considered. The technique of simultaneous excitation is especially difficult as the emission spectra of the fluorophores are very broad. This second disadvantage results in an overlap of the emission spectra and challenges the spectral separation of the emission. A third main disadvantage of traditional fluorescence molecules is the very low intensity of single molecules requiring a very sensitive detection device such as intensified cameras or avalanche photodiodes. Fourth the limited lifetime of the single molecules limits the possible observation time to the range of several seconds.

The recently introduced quantum dots (QDs) have shown to overcome the described limits of conventional chromophores. The use of QDs for colocalization has first been proposed by Lacoste et al. (2000) [7]. By using a multicolor sample-scanning confocal microscope they were able to measure the static colocalization of QDs. By using a quantum confinement effect, the emission wavelength can be tuned through almost the entire visible range while as excitation wavelength any wavelength shorter than the emission wavelength can be chosen. For instance QDs with emission peaks at 535 and 630 nm can both be simultaneously excited with any wavelength shorter than 515 nm. Additionally the quantum efficiency can considerably be increased by overcoating CdSe QDs with a few monolayers of ZnS. As the emission spectrum is very narrow (FWHM = 10–20 nm) the spectral separation is easier and photobleaching is negligible [8].

Here we show that the dynamics of the colocalization of single QDs can already be obtained with a standard optical microscope with a straightforward modification. Using a commercial inverted fluorescence microscope and a set of dichroic mirrors, we were able to measure the individual dynamics of single nanocrystals with a nanometer precision. The emission intensity from QDs excited by a mercury lamp featured a good signal-to-noise ratio.

Materials and methods

The CdSe QDs are grown by following the method of Murray, Norris, and Bawendi and overcoated by several layers of higher bandgap semiconductor ZnS [8]. The photoluminescence from these QDs features narrow emission spectra, with a span in the visible spectral range. For the experiments the nanocrystals were either diluted in Hexane and dried on cleaned cover glasses or diluted in DMPS 12M (Sigma, USA) and sandwiched between two cover glasses.

For a measurement, a commercially available inverted fluorescence microscope (DMIR, Leica, Germany) was used. As the excitation light source we used a mercury lamp (HBO 100, Osram, Germany). Two different bandpass filters were used, one with the peak excitation at 460 ± 20 nm and another one with the peak excitation 490 ± 20 nm. The emission was observed with a long pass filter with a cut off at 515 or 535 nm, respectively. The emission beam was splitted by a dichroic mirror and folded back onto one CCD chip as shown in Figure 1 (W-View, Hamamatsu, USA). We used the ORCA-ER (Hamamatsu, USA) as a camera and captured images were stored directly onto the hard disc using a recently developed imaging acquisition software OPEN BOX (Informationssysteme Schilling, Munich, Germany). The alignment of the beam splitter was done with the help of two dimensional crystal of colloidal particles dried on a cover slip. Using particle-tracking routines it was possible to align both channels with a subpixel precision over the whole field of view.

Results

In a first set of experiments the nanocrystals with emission wavelengths of 535 and 615 nm were diluted and

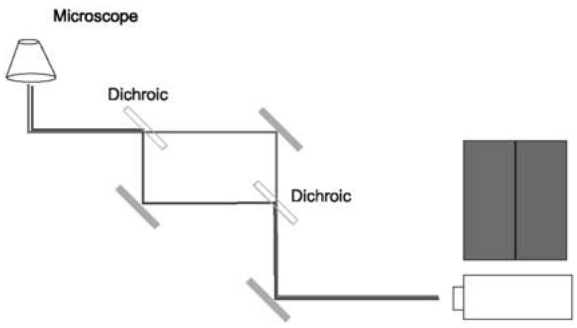

Figure 1. Schematic of the setup for the two color particle tracking. The nanocrystals are imaged with a standard fluorescence microscope. The imaging beam is split by a dichroic mirror and refolded onto single CCD chip. Consequently, in this example, on the left side of the resulting image only QDs emitting at wavelengths smaller than 590 nm are visible, whereas on the right side the nanocrystals emitting above 590 nm are visible.

mixed in Hexane and subsequently dried and immobilized on a cover glass (Figure 2). The signal-to-noise ratio of the imaged single nanocrystals was roughly 20 which allowed the determination of the position of single nanocrystals with a standard deviation of 12 nm. The beam splitting allowed the measurement of the distance between single nanocrystals well below 200 nm, in the example shown in Figure 3 the measured distance was 40 nm. The time resolution in this example was 116 ms and the total observation time was 10 min. Because of the integration time of the CCD camera only the long blinking events were observed, which was used to identify single nanocrystals. The fast blinking events were not directly observed and resulted in different intensity levels. A typical intensity profile of a single nanocrystal is shown in Figure 4. The particle tracking routine fitted a two-dimensional Gaussian to the intensity levels of a region of interest (ROI). An adjustable threshold level for the total intensity was used to stop and resume the tracking routines during the long 'off' events. Excitation with wavelengths in the UV resulted in much higher emitting intensities allowing the detection at much faster speeds. Consequently, we were able to determine the positions of single nanocrystals with a time resolution of up to 10 ms. However, excitation at these short wavelengths resulted in considerably higher photobleaching effects, which limited the observations to time periods not longer than 5 min. In a second series of experiments we diluted the nanocrystals with different emission wavelengths in DMPS and observed a shear induced flow. In Figure 5 we show that the relative motion of the different colored particles at distances well below the Rayleigh criterium can be determined. As the intensity profile of a point light source is centric and symmetric in the vertical direction, displacements in z directions do not contribute to an uncertainty in the determination of the two-dimensional projection of the particle positions. It is important to note that the dynamics of both individual QDs were determined with a high accuracy, but as a two-dimensional projection is observed the absolute distances between them cannot be determined. The only limitation of the observation time comes from the fact that during the experiment the particles drifted out of focus. This limitation could be overcome by a feedback regulation of the focal plane.

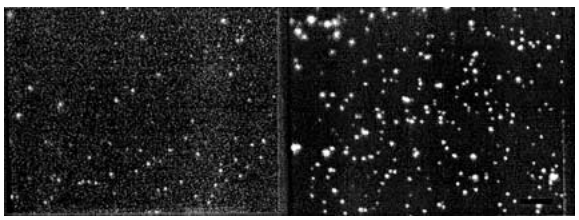

Figure 2. Experimental image of single nanocrystals with different spectral characteristic. As described in the text the image is split in the middle showing the same spatial regions but split by the wavelengths. On the left nanocrystals emitting in the green and on the right nanocrystals emitting in the red are visible. The spectral seperation of the two populations is clearly visible, thus colocalizations can be determined. The scale bar is 5 μm.

In order to take the fluorescence intermittence of the QDs into account we had to modify the automated particle tracking routines. By setting dynamically adapting intensity thresholds and region of interests in

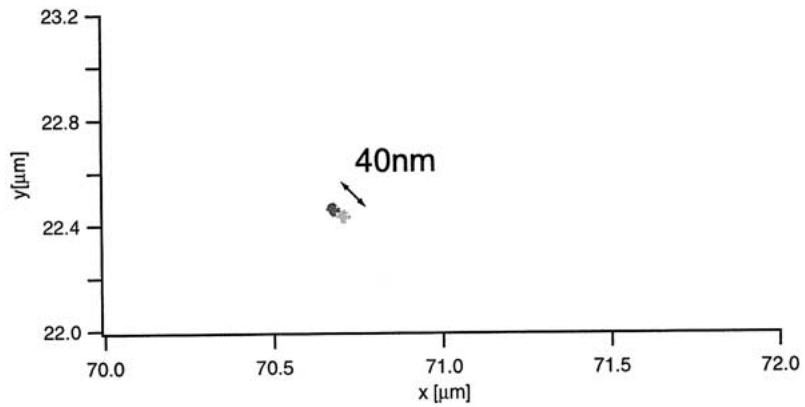

Figure 3. Particle tracking results of colocalized nanocrystals, immobilized on a cover glass with a lag time between images of 116 ms over a total observation time of 10 min. Instrumental drift is substracted from the trajectories. The measured distance between the two nanocrystals is 40 nm.

most cases we were able to track the positions automatically. In some cases, where the displacements of the QDs between two consecutive 'on times' was too big new region of interest had to be determined manually.

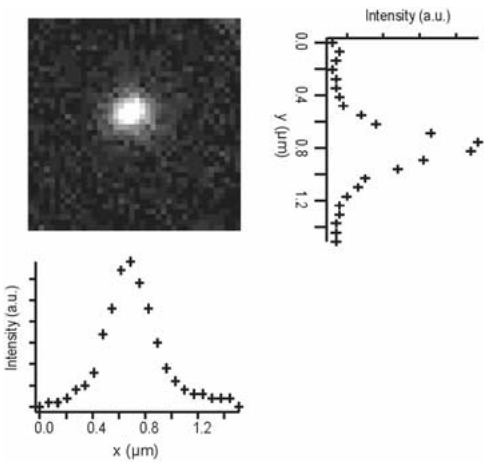

Figure 4. Diffraction limited image of a single nanocrystal. The total image covers 2.8 µm × 2.8 µm and the intensity profiles are shown.

Discussion

We have shown that it is possible to use the QDs to separate and observe the dynamics on length scales well below the optical resolution. This can be done with a nanometer precision and over different time scales ranging from milliseconds to hours. We have shown that the frequent fluorescence intermittency, albeit considered as a major disadvantage of the QDs, still allows the determination of the positions with subpixel resolution. Adapting the particle tracking routines to the blinking of the nanocrystals and the possibility of observation times up to hours opens new fields of applications. Still, for some applications where still a continuous determination of particle positions are necessary, much more work on improving the blinking of the QDs is necessary.

Until now most studies on QDs concentrated on the optical properties of the QDs. Recently different approaches for the biofunctionalization of the surface of the QDs were suggested and significant progress can be expected in the near future, which will allow the application of these particles to biological systems. It is expected that QDs will be very promising for biological studies. In parallel it is necessary to explore new

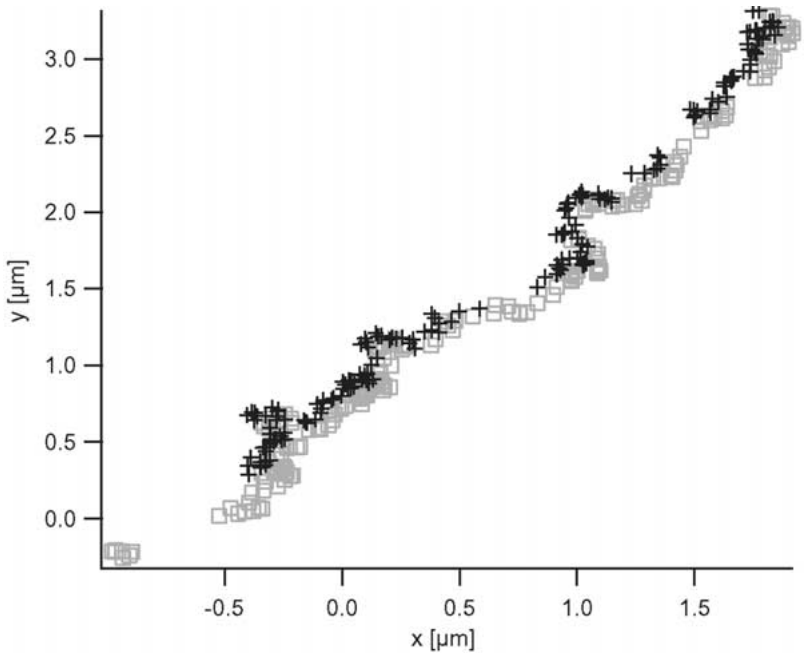

Figure 5. Trajectories of two colocalized nanocrystals during flow in DMPS. The measured distance varied between 30 and 50 nm. As the viscosity of the used solvent is very high, the diffusion of the nanocrystals is slow. Consequently, the particles stay in focus for a long time period and parallel flow of the particles is observable. Note that missing data points are due to the blinking of the nanocrystals.

experimental techniques, which use the advantages of the QDs for quantitative measurements.

For the application of the proposed technique to biological systems it is important to note that for all measurements a standard fluorescence microscope with a mercury lamp as a light source was used. This straightforward setup clearly underlines the potential for future biological studies. Because of their small size QDs are well suited to track internal protein motion without affecting the measurement. Multicolor tagging of biological molecules will offer new possibilities to observe their interaction dynamics with each other. Unlike the current static colocalization studies used in biology, this technique will open the possibility to observe the actual dynamics of proteins and their interactions over an extended time scale from tenths of milliseconds to hours.

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