Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2009 March 31; 113(27): 11541–11545. doi:10.1021/jp811078e.

Suppression of quantum dot blinking in DTT-doped polymer films

Josh Antelman^{1,+}, Yuval Ebenstein^{1,+,*}, Thomas Dertinger¹, Xavier Michalet¹, and Shimon Weiss^{1,2,3,*}

¹Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, CA 90095 USA

²Department of Physiology, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, CA 90095 USA

³DOE Institute for Genomics and Proteomics, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, CA 90095 USA

Abstract

In this report we evaluate the emission properties of single quantum dots embedded in a thin, thiol containing polymer film. We report the suppression of quantum dot blinking leading to a continuous photon flux from both organic and water soluble quantum dots and demonstrate their application as robust fluorescent point sources for ultrahigh resolution localization. In addition, we apply the polymer coating to cell samples immunostained with antibody conjugated QDs and show that fluorescence intensity from the polymer embedded cells shows no sign of degradation after 67 hours of continuous excitation. The reported thin polymer film coating may prove advantageous for immuno-cyto/histo-chemistry as well as for the fabrication of quantum dot containing devices requiring a reliable and stable photon source (including a single photon source) or stable charge characteristics while maintaining intimate contact between the quantum dot and the surrounding matrix.

The benefits of colloidal, semiconductor quantum dots (QD) as point-like fluorescent sources have long been accepted by the scientific community^{1–5}. The tunable emission wavelength and continuous absorption spectrum, along with exceptional photostability are some of the key features rendering QDs advantageous for a variety of applications from fluorescent tags to photovoltaics. Despite these advantages, many of these applications suffer from yet another common property of QDs: intermittent fluorescence known as blinking⁶. This phenomenon is observed as the turning “on” and “off” of fluorescence emission under continuous excitation of QDs. The distribution of “on” and “off” duration has been found to follow inverse power law statistics^{7,8} and various models have been suggested to explain the mechanism of QD blinking^{9–12}. Although the exact mechanism underlying this behavior is not yet entirely clear there is a consensus regarding the effect of charge on the emission state of QDs. When a charge carrier is ejected from the core (via an Auger process or charge tunneling) and trapped on its surface or in its close vicinity, the QD is thus charged and all subsequent excitons recombine non-radiatively, resulting in a “dark” QD. This lasts until the core charge is neutralized. One of the major contributors to carrier trap states is the shell surface. Charge carriers may be trapped either in surface states arising from incomplete passivation of shell surface atoms or in ligand states. Hohng and Ha¹³ have shown that the addition of 1–1000 mM of a short dithiol molecule such as β -mercaptoethanol (BME) results in an almost complete suppression of blinking. It has been suggested that small, thiol containing molecules which are mobile electron

*corresponding authors: Email: uv@che.ucla.edu, sweiss@chem.ucla.edu.

⁺These authors contributed equally to this work.

donors serve to eliminate surface electron traps, preventing their availability to QD core electrons. This is in line with the observation that thiols had no effect on “off” time statistics (representing transitions from “off” to “on” due to release of a trapped carrier and neutralization of the core) but considerably extended the duration of “on” times, indicating a reduced probability of core charge carriers to be trapped and lead to an “off” state¹³. Recently, a non thiolated small ligand (propyl gallate) has also been reported to suppress blinking of QDs in aqueous solution¹⁴. This observation indicates that blinking suppression is not thiol specific but related to the occupation of trap sites on the shell surface. Although the addition of such small ligand molecules to QD suspensions remarkably suppresses blinking, it requires the QDs to be constantly immersed in the ligand solution. This poses limitations on the kind of experiments and applications possible with these QDs. Solid state devices and many microscopy techniques require that the QD be embedded in a solid matrix, usually a thin polymer film. Such films have been shown to allow observation times of hours for single QDs but with pronounced blinking^{3,15}. Recently, two groups have reported the synthesis of QDs consisting of a core overcoated with a thick crystalline shell of a higher bandgap semiconductor^{16,17}. These QDs are reported to display considerably reduced blinking without the need for extra surface passivation steps. This improvement, however, comes at the expenses of larger size, and a large potential barrier between the core and its surrounding environment. Some applications require a controlled way of transferring charges between the QD core and the surrounding matrix (rather than uncontrolled charge trapping at the surface) and a thick barrier will impede this. The performance of QD containing devices such as current-driven (electrically pumped) light emitting diodes and solar cells is directly related to the efficiency of this charge transfer. The ability to suppress blinking of matrix embedded QD while keeping a relatively low potential barrier between the core and its surroundings is therefore desirable.

A powerful method for studying single QDs utilizes stage-scanning confocal microscopy. This method employs a piezo-scanner to raster scan a sample of immobilized QDs over a diffraction-limited excitation spot. Emitted photons are detected pixel-by-pixel to create a raster-scanned image representing the localization of the QDs dispersed on the substrate. The diameter of the resulting fluorescence spots mirrors the dimension of the excitation spot and therefore is limited by diffraction. The corresponding point-spread-function (PSF) has a typical diameter of 300 nm-500 nm depending on the excitation wavelength. By fitting the acquired PSF to a 2-dimensional Gaussian, the center of the PSF, representing the exact location of the imaged QD may be determined with great precision^{3,18}. Unfortunately, blinking of the QD during image acquisition results in a characteristic streaking of the PSF as seen in Figure 1a. Streaking reduces the integrity of the PSF and therefore the quality of the Gaussian fit, reducing the localization accuracy. Furthermore, blinking reduces the total number of photons per PSF, therefore reducing the achievable accuracy¹⁹. We have previously shown that, using a stage-scanning confocal setup, the distance between two QDs of different color may be measured down to less than 25 nm with an uncertainty σ of 13 nm²⁰. As we will show, this uncertainty can be reduced extensively in the absence of blinking.

Here we present a simple method to suppress QD blinking by encapsulation in a thin polymer film containing dithiothreitol (DTT). The embedded QDs exhibit unprecedented photostability under prolonged excitation. Blinking of the QDs is highly suppressed resulting in a continuous and stable photon flux.

Figure 1a shows a stage-scanning confocal image of commercially available streptavidin-conjugated QDs emitting at 655 nm (Qdot 655, Invitrogen) that were spin-cast from buffer onto a glass coverslip and coated by a polyvinyl alcohol (PVA) film. 10–100 μ l of a 10 mg/ml, PVA/water solution was spin-cast on top of the QDs at 6000 rpm, and left to spin for 50 seconds to allow complete drying of the polymer. The two-step deposition of QDs and polymer used here was convenient in this case since it allowed us to tune the surface density of QDs

and confine them to the coverslip plane before application of the polymer coating. Images were acquired using a custom built stage scanning microscope equipped with a high numerical aperture objective (60X Plan-Apo, NA 1.45, Olympus). For excitation, the 488 line of an air cooled Argon ion laser was used with an incident power of 230 nW at the back focal plane of the objective lens. Fluorescence was detected by a single-photon avalanche photodiode (SPAD) (SPCM-14, PerkinElmer). The streaked features of the imaged PSFs in Figure 1a are a clear signature of blinking. In this case, every pixel is integrated for a period of 25 ms and an off time longer than this integration time will result in one or more consecutive dark pixels as the QD is scanned through the excitation spot. The extent of blinking is emphasized in the time trace recorded for one of these QDs by parking it over the excitation spot and recording its emission as a function of time using the same excitation power and detection optics used for the scanned images. A typical time trace for this sample is presented in Figure 1c. To eliminate blinking, 80mg/ml DTT (Research Products International) was added to the PVA solution and spin cast over a QD sample as described above. A remarkable effect on blinking is evident from Figure 1b and 1c. The streaked pattern of the PSF is replaced by a smooth Airy disk distribution, and the time trace shows very rare blinking events. A 20 minutes long temporal emission pattern of a single QD is shown in Figure 2. A gradual intensity decrease was observed over the ~ 20 min time trace duration and was attributable to sample drift across the confocal excitation spot. Indeed, fluorescence intensity observed by wide field imaging on a CCD (in which lateral drift has no effect on the recorded intensity) shows no significant decrease in signal intensity on this time scale. For this reason, the data was corrected for drift using a linear fit to the measured time trace and applying a time dependent correction factor to compensate for loss of signal along the measurement. A histogram of the intensity levels for this QD emphasizes the suppression of blinking, with the majority of the data concentrated around the mean value of 36 kHz. By defining the “off” state as being below two standard deviations from the mean intensity, we find that the QD is “on” for 94 % of the time.

To demonstrate the general applicability of this method, we applied the coating to a variety of other QD preparations including similar streptavidin-conjugated QDs emitting at 585nm (Qdot 585, Invitrogen). The performance of these QDs was even better than that of the previous 655 nm emitting QDs, and resulted in a very stable emission pattern indicating that even “off” times shorter than our 10 ms time bin are largely suppressed. Another QD preparation of interest is the organic, Trioctylphosphine oxide (TOPO) capped QDs. For the sake of consistency, we present data on commercially available QDs identical to the streptavidin-conjugated QDs presented above but lacking the bio-compatible polymer coating (Qdot 655 organic, Invitrogen). Figure 3 shows typical time trajectory images for such particles with, and without addition of DTT to the PVA coating. Movies were acquired using an EMCCD (DU897, Andor) in frame transfer mode with 20 ms integration time. A region with no overlapping particles along the Y axis has been chosen and an orthogonal slice view of this region is displayed (IQ, Andor). The trajectory image displays the integrated signal from the corresponding line in a single frame such that the full trajectory represents the temporal emission of the QD during the acquisition time. Clearly, Blinking has been significantly eliminated for most QDs relative to the QD embedded in PVA not containing DTT.

Since many applications require the use of nonpolar polymers dispersed in organic solvents we have successfully achieved similar results using non-polar, trioctyl phosphine (TOPO) capped QDs (Qdot 655 organic, Invitrogen) dispersed in 10mg/ml Zeonex in chloroform. Zeonex (Zeonex E48R, Zeon corp.) is a cyclo-olefin polymer (polynorbornene) with exceptional optical properties and extremely low moisture absorption. Polymethyl methacrylate (PMMA), polystyrene (PS) and Zeonex are commonly used to enhance the photostability of single QDs and other fluorophores by embedding them in the polymer matrix. In order to introduce DTT into the Zeonex matrix, a co-solvent such as ethanol is used to solubilize DTT (100 mg/ml) which is then mixed into the Zeonex-chloroform solution in a 1:5,

ethanol:chloroform ratio. Upon addition of ethanol-DTT, a white precipitate was visible. The solution was centrifuged for a few seconds and 10–100 μL of the t, QD containing phase was spin cast onto a clean coverslip at 3700RPM. Figure 4 shows Zeonex-DTT coated QD samples at various time points. Interestingly, the QD fluorescence signal was undetectable or weak to start with, but it constantly increased under continuous excitation until it stabilized after about 3 minutes (Fig 4a and 4b). Similar behavior has been observed for QDs treated for fluorescence in situ hybridization (FISH)²¹ and may be caused by light induced heating of the polymer allowing diffusion of DTT molecules onto the QD surface. QD diffusion was observed after a few minutes of continuous excitation as clearly shown in the trajectory images (displacement of QDs along the Y axis. The images summing all frames show broadening of the area occupied by the QD relative to the single frame images (Figure 4c,e and 4d,f). Most likely, one or more QDs are trapped within quasi-circular cavities created in the polymer, presumably due to heating during excitation. Nevertheless, the QDs remained bright and exhibited reduced blinking. In fact, these conditions allowed us to observe the particles after 20 hours of continuous excitation with no significant periods of blinking or reduction of signal intensity (Fig. 4d). Although we provide no direct evidence that the observed emission is generated from single QDs, it is clear that particles cease to blink upon application of the coating. Blinking is commonly used to assess the “singleness” of a QD emitter; this is no longer applicable for the treated non-blinking samples. Photon antibunching^{22–24} as well as spectral²⁵ and lifetime analysis^{26,27} of these samples will address this issue more carefully in future work. We believe that this approach could be applied to a variety of other polymers including conducting and semi-conducting polymers used in the fabrication of photonics devices. It is also possible that treated QDs will function as reliable single photon sources for quantum computing applications^{28,29}.

To demonstrate the effect of blinking suppression on the quality of high-resolution localization measurements of single QDs, we performed bootstrap analysis on single QD signals acquired from untreated vs. treated, non blinking QDs. 1 μm^2 scans consisting of 80x80 pixels of isolated QD fluorescence signals were acquired at 10 ms per pixel with an excitation power of 0.3 – 2.0 μW (tuned to produce equivalent fluorescence signal between the different QDs). A relatively “well behaved” blinking QD was chosen for the comparison as presented in Figure 5a and 5b along with cross sections in X and Y through the Gaussian center and the corresponding fitted Gaussians. Ideally, multiple measurements must be evaluated in order to determine the real experimental error in position resolved by the fit. However, such an approach is not practical for this experimental configuration and therefore we use a bootstrap approach, which allows us to fit a random subset of the data numerous times to assess the reliability of the measurement and determine the likely positional error of the fit^{3,30}. A perfect fit will yield similar values regardless of the subset used while non ideal data will skew the fit result depending on the subset of data used. The width of the distribution of the resulting fitted centers gives a good estimate of the localization uncertainty. Figure 5b and 5c show the distribution of centers derived from 1,000 bootstrap simulations and their Gaussian fits. This analysis yielded an average position error of 1.7 nm for the untreated QD vs. 0.4 nm for the QD embedded in the polymer-DTT matrix. This result corresponds to a 4-fold improvement in resolution enabling real sub-nm localization.

The long term fluorescence stability exhibited by the treated QDs (as seen in Figure 4 d) implies that the DTT doped polymer coating can provide exceptional encapsulation for QD samples. We have tested the potential of our polymer coating for preservation and archiving of biological samples. Several reports have already demonstrated the superior fluorescence stability of QDs over organic dyes in immunofluorescence labeling of cells and tissue samples^{31–34}. We studied the photostability of immunolabeled microtubules in fixed 3T3 cells and observed a dramatic improvement in photosability after applying the polymer coating to the QD labeled sample (Figure 6). 3T3 mouse fibroblast cells were plated in LabTek coverslide chambers. After 24–

48 h, the cells were fixed for 15 min (3.7 % formaldehyde, 0.5% glutaraldehyde, 5% sucrose, 0.25% Triton X 100 in 10mM MES pH 6.2, 140 mM NaCl). The fixative was quenched for 8 min (10mM MES pH 6.2, 140 mM NaCl, 0.5 mg / ml Sodium borohydride) and fixed cells were washed with PBS and permeabilized in blocking buffer (PBS containing 2 % w/v bovine albumin serum (BSA; Sigma)) for several hours. Microtubules were stained with mouse monoclonal anti- α -tubulin antibodies (Abcam) for 60 min. Primary antibodies were either FITC conjugated or further labeled with a secondary anti-mouse–QD conjugate (Qdot 625, Invitrogen) following the manufacturers' protocol. FITC and QD labeled samples in PBS were imaged for 20 min and then the QD labeled sample was washed with ethanol and air dried. 50 μ l of PVA-DTT were spin cast over the sample followed by 50 μ l of zeonex solution and the sample was subjected to fluorescence imaging. All samples were excited with a 75 W Xenon arc lamp through a 30 nm bandpass filter centered at 470 nm. Images of the three samples at $t = 0, 5$ and 250 seconds are displayed in Figure 6a, 6b and 6c. The image at $t = 0$ was optimized for contrast and the same setting was used to display the other images. Figure 6d, shows the progression of fluorescence intensity with time under continuous excitation. Mean intensity values were calculated from regions of interest in the image and normalized for comparison. A first order exponential fit to the three curves yielded a time constant of 2721 s, 326 s and 3.7 s for the treated QD sample, the untreated QD sample and the FITC sample respectively. While fluorescence intensity from un-treated QDs and FITC labeled cells decayed with time in accordance with the fitted time constant, the polymer embedded sample increased slightly in fluorescence intensity after 67 hours of continuous excitation by a 75 W (470/30) Xenon lamp. This preliminary observation is promising although a more thorough investigation of the preservation properties of the polymer coating compared to other archiving media is necessary in order to determine its' advantages.

In conclusion, a simple method for the preparation of a DTT containing polymer matrix was presented. When used to coat QDs, the polymer coating serves to suppress QD fluorescence blinking and to protect the QDs against photodegradation. This method has allowed us to observe QD immunolabeled microtubules as well as single QDs for prolonged periods under continuous excitation. Fluorescence fluctuations were considerably reduced and in some cases completely eliminated. We demonstrated the enhanced position accuracy achieved by localizing non-blinking vs. blinking QDs and the potential of the coating as an archiving medium for immuno-cyto/histo-chemistry. . We also envision potential applications for this approach in thin film based devices containing QDs such as LEDs^{35,36} or photovoltaic cells^{37,38} and as in quantum computing and other “single photon demand” applications^{22,23, 28,39}.

Acknowledgments

We thank Invitrogen for the generous gift of Qdots and Zeon Corp for the generous gift of Zeonex. This work was supported by the UCLA-DOE Institute for Genomics and Proteomics (grant DE-FC02-02ER63421) and NIH grant R01-EB000312. Y.E thanks the Human Frontier Science Program for their support. T. D. thanks the German Science Foundation (DFG) for financial support (DE 1591/1-1).

REFERENCES

1. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Science 2005;307:538. [PubMed: 15681376]
2. Alivisatos AP. Journal of Physical Chemistry 1996;100:13226.
3. Michalet X, Pinaud F, Lacoste TD, Dahan M, Bruchez MP, Alivisatos AP, Weiss S. Single Mol 2001;2:261.
4. Alivisatos AP. Science 1996;271:933.
5. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Nat Mater 2005;4:435. [PubMed: 15928695]

6. Nirmal M, Dabbousi BO, Bawendi MG, Macklin JJ, Trautman JK, Harris TD, Brus LE. *Nature* 1996;383:802.
7. Shimizu KT, Neuhauser RG, Leatherdale CA, Empedocles SA, Woo WK, Bawendi MG. *Phys. Rev. B* 2001;63(205316)
8. Kuno M, Fromm DP, Hamann HF, Gallagher A, Nesbitt DJ. *J. Chem. Phys* 2000;112:3117.
9. Cichos F, von Borczyskowski C, Orrit M. *Current Opinion in Colloid & Interface Science* 2007;12:272.
10. Efros AL, Rosen M. *Physical Review Letters* 1997;78:1110.
11. Verberk R, van Oijen AM, Orrit M. *Physical Review B* 2002;66
12. Frantsuzov P, Kuno M, Janko B, Marcus RA. 2008;4:519.
13. Hohng S, Ha T. *Journal of the American Chemical Society* 2004;126:1324. [PubMed: 14759174]
14. Fomenko V, Nesbitt DJ. *Nano Letters* 2008;8:287. [PubMed: 18095736]
15. Shen Y, Pang L, Fainman Y, Griswold M, Yang S, Butov LV, Sham LJ. *Phys. Rev. B* 2007;76
16. Chen Y, Vela J, Htoon H, Casson JL, Werder DJ, Bussian DA, Klimov VI, Hollingsworth JA. *Journal of the American Chemical Society* 2008;130:5026. [PubMed: 18355011]
17. Mahler B, Spinicelli P, Buil S, Quelin X, Hermier J-P, Dubertret B. *Nature Materials*. 2008 *advanced online publication*.
18. Thompson RE, Larson DR, Webb WW. *Biophys. J* 2002;82:2775. [PubMed: 11964263]
19. Michalet X, Lacoste TD, Weiss S. *Methods* 2001;25:87. [PubMed: 11559000]
20. Lacoste, TD.; Michalet, X.; Pinaud, F.; Chemla, DS.; Alivisatos, AP.; Weiss, S. Vol. Vol. 97. 2000. Ultrahigh-resolution multicolor colocalization of single fluorescent probes; p. 9461
21. Bentolila LA, Weiss S. *Cell Biochem Biophys* 2006;45:59. [PubMed: 16679564]
22. Michler P, Imamoglu A, Mason MD, Carson PJ, Strouse GF, Buratto SK. *Nature* 2000;406:968. [PubMed: 10984045]
23. Lounis B, Bechtel HA, Gerion D, Alivisatos P, Moerner WE. *Chemical Physics Letters* 2000;329:399.
24. Zwiller V, Blom H, Jonsson P, Panev N, Jeppesen S, Tsegaye T, Goobar E, Pistol M-E, Samuelson L, Bjork G. *Applied Physics Letters* 2001;78:2476.
25. Neuhauser RG, Shimizu KT, Woo WK, Empedocles SA, Bawendi MG. *Physical Review Letters* 2000;85:3301. [PubMed: 11019326]
26. Schlegel G, Bohnenberger J, Potapova I, Mews A. *Physical Review Letters* 2002;88:137401. [PubMed: 11955124]
27. Labeau O, Tamarat P, Lounis B. *Physical Review Letters* 2003;90:257404. [PubMed: 12857165]
28. Santori C, Fattal D, Vuckovic J, Solomon GS, Yamamoto Y. *Nature* 2002;419:594. [PubMed: 12374958]
29. Hijlkema M, Weber B, Specht HP, Webster SC, Kuhn A, Rempe G. *Nat Phys* 2007;3:253.
30. Efron, B.; Tibshirani, RJ.; Efron, B. *An Introduction to the Bootstrap*. Vol. Vol. 57. CRC Press; 1994.
31. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. *Nat Biotech* 2003;21:47.
32. Tokumasu F, Dvorak J. *Journal of Microscopy* 2003;211:256. [PubMed: 12950474]
33. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP. *Nat Biotech* 2003;21:41.
34. Xing Y, Chaudry Q, Shen C, Kong KY, Zhau HE, Chung LW, Petros JA, O'Regan RM, Yezhelyev MV, Simons JW, Wang MD, Nie S. *Nat. Protocols* 2007;2:1152.
35. Colvin VL, Schlamp MC, Alivisatos AP. *Nature* 1994;370:354.
36. Tessler N, Medvedev V, Kazes M, Kan SH, Banin U. *Science* 2002;295:1506. [PubMed: 11859189]
37. Huynh WU, Dittmer JJ, Alivisatos AP. *Science* 2002;295:2425. [PubMed: 11923531]
38. Klimov VI. *Annual Review of Physical Chemistry* 2007;58:635.
39. Brunel C, Lounis B, Tamarat P, Orrit M. *Physical Review Letters* 1999;83:2722.

Figure 1.

Effect of PVA-DTT coating on the temporal fluorescence behavior of commercial polymer coated, water soluble QDs (Qdot 655 streptavidin, Invitrogen). **a, b:** Confocal images of PVA-coated vs PVA-DTT-coated QDs (60 nm / pixel, 25 ms / pixel, scale bar: 1 μm). **c:** Time traces of a PVA coated (black) vs PVA-DTT coated (red) QD with 100 ms time bins (top) and 25 ms time bins (bottom)

Figure 2. 20 min time trace of a PVA-DTT coated QD with 100 ms time bins acquired in Confocal mode. **Inset:** Histogram of time trace intensity values. The QD is on 94% of the time.

Figure 3.

Effects of PVA-DTT on the temporal fluorescence behavior of non-polar, TOPO coated QDs (Qdot 655 organic, Invitrogen). **a, c:** Sum image of all frames showing the locations of fluorescence spots. Image is inverted such that dark indicates bright intensity, whereas white indicates no emission. **b, d:** Compact representation of the corresponding intensity time traces obtained by projecting each frame image on the vertical axis y , resulting in a single vertical dotted segment per frame (scale bar: 10 s) **a, b:** no DTT added to the PVA coating. **c, d:** PVA-DTT coating (some spots have been shifted along the vertical axis to maintain the frame size presented in a. 4500 frames, 20 ms per frame, 470 nm excitation.

Figure 4. Nonpolar, Zeonex-DTT coating. Series of sum images of all 100 ms frames (left of each panel) showing QD locations and their corresponding intensity time traces (right of each panel) (scale bar: 10 s.). **a:** $t = 0$, onset of fluorescence. **b:** $t = 15$ min, stabilization of emission. **c:** $t = 40$ min, stable emission with some confined diffusion. A single frame is shown to the left of the sum image for comparison. **d:** $t = 20$ hours, stable emission with pronounced diffusion in defined circular regions. A single frame is shown to the left of the sum image for comparison.

Figure 5.

Localization of QD emitters. **a, b:** $1\ \mu\text{m}^2$ scans of PVA (a) and PVA-DTT (b) coated QDs and their intensity cross sections through the Gaussian center. **c:** distribution of bootstrap results for image a. **d:** distribution of bootstrap results for image b. **e:** Gaussian fit for c ($\sigma_x = 1.75\ \text{nm}$, $\sigma_y = 1.68\ \text{nm}$). and the corresponding Gaussian fit for image b ($\sigma_x = 0.44\ \text{nm}$, $\sigma_y = 0.41\ \text{nm}$). (scale bar 5 nm).

Figure 6.

Photostability of immunolabeled microtubules in fixed 3T3 cells. **a:** Microtubules were immunolabeled with secondary QD-antibodies and imaged after drying and polymer coating. Images were recorded at time points: $t = 0$ s, 10 s and 250 s. (scale bar 10 μm). **b:** Microtubules were immunolabeled with secondary QD-antibodies and imaged in PBS. **c:** Microtubules were immunolabeled with primary FITC-antibodies and imaged in PBS. **d:** Normalized plots of intensity as a function of time for the three samples.