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The Influence of Continuous vs. Pulsed Laser Excitation on Single Quantum Dot Photophysics

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Abstract

The impact of pulsed versus continuous wave (cw) laser excitation on the photophysical properties of single quantum dots (QDs) has been investigated in an experiment in which all macroscopic variables are identical except the nature of laser excitation. Pulsed excitation exaggerates the effects of photobleaching, results in a lower probability of long ON fluorescence blinking events, and leads to shorter fluorescence lifetimes with respect to cw excitation at the same wavelength and average intensity. Spectral wandering, biexciton quantum yields, and power law exponents that describe fluorescence blinking are largely insensitive to the nature of laser excitation. These results explicitly illustrate important similarities and differences in fluorescence dynamics between pulsed and cw excitation, enabling more meaningful comparisons between literature reports and aiding in the design of new experiments to mitigate possible influences of high photon flux on QDs.

Introduction

Single molecule spectroscopy (SMS) has emerged as a powerful tool to investigate quantum dot (QD) photophysics. Because the optical properties of QDs are strongly size-dependent, spectroscopic peaks are frequently broadened by the distributions of particle sizes, shapes, or local environments that exist in traditional ensemble measurements. By sampling only one QD at a time, SMS fundamentally eliminates such inhomogeneous broadening and can reveal important fundamental properties (eg, fluorescence linewidths, polarization properties, etc).[1] In addition, dynamic fluctuations in QDs' optical properties, such as fluorescence intermittency[2] and spectral wandering,[3,4] can also be observed by single molecule detection techniques. Insights to QD photophysics gained from these uniquely single molecule phenomena have led to the design of QDs with optimized optical properties and are expected to improve their use in various optical technologies.[5]

The exquisite sensitivity of single QD experiments can also create challenges when comparing measurements done under different experimental conditions. A variety of experimental variables: excitation wavelength, excitation intensity, pulsed versus continuous wave (cw) excitation, QD size, QD composition, QD surface ligand, ambient environment, local dielectric environment, and substrate, could potentially impact measurement results, and direct comparisons between different experiments are often difficult. One of the most intensively investigated single QD phenomena in the last 20 years has been fluorescence intermittency, also known as blinking. Blinking describes the phenomenon in which QDs randomly cycle between bright, emissive ON states and dark, non-emissive OFF states, all while under continuous excitation.[2] QD blinking is known to follow inverse power law statistics under a broad set of experimental

conditions.[6-8] However, important differences in blinking behavior resulting from experimentally controllable variables are also known. For example, blinking behavior can be reduced, to the benefit of most applications, both by control of the environment[9-11] and engineering of the QD structure.[12-16] Blinking is also sensitive to both the energy and intensity of the excitation source. Higher energy (shorter wavelength) excitation impacts the ON time blinking statistics[17] and contributes to fluorescence decay, blue-shift, and peak broadening.[18] High intensity excitation results in a deviation from typical power law statistics, with truncation occurring at shorter times with increasing intensities.[19-22] When using pulsed lasers as excitation sources, higher pulse rates have been demonstrated to decrease the fluorescence lifetime of QDs, with high exciton populations favoring fast nonradiative processes.[23]

Many of these changes in blinking behavior have been linked, both experimentally and theoretically, to the formation of multiple excitons.[19,22,24] Under typical experimental conditions, pulsed and cw excitation can produce very different exciton populations and one may expect differences in blinking behavior. In the case of pulsed excitation, one considers the average number of excitons per pulse, given by

$$\langle N \rangle_{pulsed} = \ln 2 \frac{P}{E_{photon} \Gamma} \frac{\sigma_{abs}}{\pi r^2}$$

where P is the average laser power, E_{photon} is the photon energy, Γ is the laser repetition rate, σ is the absorption cross-section, and r is the radius of the focused laser beam at half its maximum intensity. In the case of cw excitation, one considers the average number of excitons per fluorescence lifetime, given by

$$\langle N \rangle_{cw} = \ln 2 \frac{P \tau_{fluor}}{E_{photon}} \frac{\sigma_{abs}}{\pi r^2}$$

where τ_{fluor} is the fluorescence lifetime. In an experiment in which all variables are the same except the nature of excitation, typical experimental conditions (Γ = 2.5 MHz, τ_{fluor} = 25 ns) produce approximately 15 times more excitons in the case of pulsed versus cw excitation. The population of multiexcitons scales nonlinearly with power, and thus the differences between cw and pulsed laser sources are even more dramatic. Pulsed excitation would be expected to produce more than 150 times more biexcitons than cw excitation under the same experimental conditions described above.[19]

This example highlights the need to carefully understand the impact of pulsed versus cw excitation sources in single QD fluorescence studies. QD fluorescence dynamics have been investigated in an experiment in which all macroscopic experimental variables were identical, except for the nature of the laser excitation. Metrics studied include: (1) blinking statistics (ON/OFF probability density vs. ON/OFF duration), (2) photobleaching time, (3) fluorescence lifetime dynamics, (4) spectral diffusion, and (5) photon antibunching. The results demonstrate that pulsed versus cw excitation does impact the fluorescence dynamics and helps clarify the impact of the nature of laser excitation on important single QD phenomena.

Experimental

Commercial QDs, Invitrogen organic capped QDs with 585 nm emission maximum (QDot585), were chosen for the sake of facile reproducibility and ease of comparison to future experiments. Although Invitrogen does not provide an exact composition of their QDot products for proprietary reasons, the sample is known to have a CdSe/ZnS core/shell structure with an additional polymer layer for passivation.[25]

Sample preparation details are provided in the Supporting Information. Briefly, the CdSe QDs were precipitated through the addition of a non-solvent, centrifuged into a pellet, resuspended in toluene, and diluted with 0.1% solution of polystyrene in toluene. A spin-coater was used to create a polymer and QD film on a quartz microscope slide. Single-molecule fluorescence was collected with an inverted microscope (Nikon TU-3000), and a LabView–controlled scanning stage (Mad City Labs NanoBio100) for confocal collection. A diagram of the apparatus and further instrument details are provided in the Supporting Information as Fig. SI.1. Excitation was provided by a 485 nm diode laser (PicoQuant PDL 800-D with LDH-D-C-485 laser head), which switches between cw and pulsed operation without any significant change in shape or alignment of a focused laser spot, and less than a 5-nm shift in excitation wavelength. The laser beam was focused to a 320-nm diameter spot by a 100× Plan Fluor oil immersion objective (NA = 1.4). Pulsed excitation was performed with a repetition rate of 2.5 MHz. Power levels were attenuated by neutral density filters so that average power was ~800 nW for both pulsed and cw excitation, corresponding to an average intensity of 1000 Wcm^{-2}. For pulsed excitation, a QD was exposed to 7.7×10^5 incident photons in each pulse. For cw, we consider 4.8×10^4 photons within the ~25 ns fluorescence lifetime of the QD. The resulting average numbers of excitons are $\langle N \rangle_{pulsed} = 2.7$ and $\langle N \rangle_{cw} = 0.17$.

Excitation light was removed from the emission path with a Chroma dichroic beamsplitter and two Semrock “Razor Edge” long-pass filters. Fluorescence was detected by an avalanche photodiode (APD, Micro Photon Devices PDM Series 5CTB) with a PicoHarp300 counting card (Picoquant GmbH) for time-tagged time-resolved (TTTR) data. This photon counting system tags each photon with arrival times defined

with respect to the beginning of the collection window (for fluorescence intensity trajectories to observe blinking behavior), and in the case of pulsed excitation, with respect to the most recent laser pulse (for photon arrival time histograms to determine fluorescence lifetimes). The resolution of the counting card was 128 ps. Photon counts obtained in TTTR mode were binned in 100 ms increments to construct fluorescence intensity trajectories. Fluorescence was collected for 20 minutes for each QD studied, over 100 in total (60 cw and 43 pulsed).

Blinking dynamics were analyzed using threshold analysis. The threshold was selected at $I_{off} + 2\sigma$, where I_{off} is the most probable and σ is the standard deviation of OFF state intensities obtained from a histogram of all counts in a given trace. Probability densities[19,26] of event durations were calculated according to equation

$$P(\tau_i) = \frac{N(\tau_i)}{N_{tot}} \frac{2}{\tau_{i+1} - \tau_{i-1}}$$

where $N(\tau_i)$ is the number of events of a duration τ_i, N_{total} is the total number of events, and the second term is the average duration between preceding and succeeding event times (*ie,* the average time bin width). Events from 60 QDs for cw excitation and 43 QDs for pulsed excitation were aggregated together for analysis. The statistical parameters determined from analyzing aggregated blinking data are within experimental uncertainties of those determined from analyzing individual blinking data sets and provide the necessary sample size for robust statistics.[26,27]

Fluorescence lifetime is determined by fitting the TTTR data with a single exponential in 2 s bins. In some cases a single exponential does not fully describe the lifetime curves and the quality of the fit can be improved using a double exponential function (such an example is apparent in Fig. 2a). However, the change in the

fluorescence lifetime obtained using double exponential fitting was minimal (typically <5%) compared to the QD-to-QD variation (typically 20%). Furthermore, because we are primarily interested in differences between cw and pulsed excitation and because any small systematic differences in fitting routines are expected to be distributed equally between pulsed and cw data sets, we used the simplest fitting routine (*i.e.,* single exponential fitting) that allowed consistent comparisons among data sets. Single molecule fluorescence spectra were collected with a liquid nitrogen-cooled charge coupled device (Princeton Instruments Spec-10). Spectra were collected with 1 minute integration times, sequentially for 10 minutes for each QD (12 excited by cw, and 10 excited by pulsed). Second-order emission intensity correlation function $g^{(2)}$ measurements were made in a Hanbury-Brown-Twiss geometry. The relative arrival times between two successive photons were recorded by the Picoharp 300 photon counting card with a 128 ps timing resolution. The data was analyzed following reference 28.

Results and Discussion

Sample fluorescence intensity time traces obtained with cw and pulsed excitation are shown in Figs. 1a and 1b. The cw and pulsed intensity time traces are qualitatively similar in many regards. The ON intensities are within ~30%, as expected for equal average excitation powers. ON and OFF events of various durations are observed for both excitation schemes. However, one striking qualitative difference is the gradual loss of fluorescence intensity that is observed in the pulsed excitation trace (see also supporting information Fig. SI.2). Although its exact origin is unknown, such

irreversible decreases in fluorescence intensity commonly are associated with photo-induced degradation of the QD surface. 70% (30 of 43) of the pulsed traces exhibited this intensity loss on the 20 min timescale of the current experiment, compared to 57% (34 of 60) of the cw traces. The half-lives for this process (ie, the time required for the fluorescence intensity to reach half its initial value, or to photobleach completely if that occurred first) spanned a wide range of values. The average half-lives and standard deviations for those QDs exhibiting measureable half lives were 660 ± 370 s and 510 ± 260 s for cw and pulsed, respectively (excluding the 10 cw and 2 pulsed traces which neither exhibit an intensity decrease nor photobleach).

Analysis of blinking dynamics shows differences between pulsed and cw excitation. The probability distributions for ON and OFF events are shown in Figs. 1c and 1d. The OFF and ON distributions are fit to either a power law or modified power law,[19, 26,29] of the forms

$$P(\tau_{OFF}) = A\tau_{OFF}^{-m_{OFF}}$$

$$P(\tau_{ON}) = A\tau_{ON}^{-m_{ON}} e^{-\tau_{ON}/\tau_0}$$

where A and m are constants, and τ_0 describes the characteristic time at which deviations from the power law behavior begin (sometimes referred to as a "falloff" time). The choices of both bin size and threshold level have been shown to affect the statistical analysis of ON and OFF times.[26] However, we are primarily interested in differences between pulsed and cw excitation schemes and since our methods were applied consistently for the cw and pulsed data, comparisons are valid. Furthermore, the use of an aggregated data set provides a sufficiently large number of events (*i.e.,* over 11,000

events for both cw and pulsed) that analysis artifacts are minimized and robust statistics can be obtained.[26,27]

The power law exponents obtained with pulsed and cw excitation are within experimental uncertainties for both OFF and ON distributions m_{OFF} values are 1.71 ± 0.02 and 1.79 ± 0.02 for pulsed and cw excitation respectively; m_{ON} values are 1.34 ± 0.04 for cw and 1.49 ± 0.06 for pulsed excitation. However, the values for τ_0 are significantly different, 44 ± 3 s for cw excitation and 20 ± 2 s for pulsed excitation. The larger falloff time for cw excitation corresponds to higher probabilities of long-lived ON events for cw compared to pulsed excitation. The fall-off time has been associated with the formation of biexcitons.[19] In the same time period, ~10 times more biexcitons are produced in the case of pulsed excitation compared to cw-excitation, therefore, the smaller falloff time is expected for pulsed excitation.

We have also performed control experiments in which the laser power is varied such that the average exciton population, rather than the average power, is the same under cw and pulsed excitation. We find that at low exciton populations ($\langle N \rangle < 1$) the inverse of the fall-off time τ_0^{-1} scales linearly with the average exciton population (Fig. 1e). Furthermore, the slopes of τ_0^{-1} versus $\langle N \rangle$ are similar for both pulsed and cw excitation, suggesting a common origin for the fall-off behavior in both excitation modes (*e..g,* multiexciton species). At higher exciton populations ($\langle N \rangle \sim 2$) τ_0^{-1} saturates, reaching a maximum of ~ 0.1 s^{-1}, consistent with previous reports.[22] Other blinking parameters (eg, ON and OFF power law slopes) are independent or only weakly dependent on the exciton population.

Comparison of fluorescence lifetime data also shows subtle differences between pulsed and cw excitation. Unfortunately, the nature of the experiment does not allow direct comparison of QD fluorescence lifetimes under both excitation types, as the laser pulse is necessarily required by the photon counting system as a reference point for lifetime calculations. To approximate the influence of cw excitation on the fluorescence lifetime of the QDs, we designed an experiment in which we exposed the QD to cw excitation for a set amount of time (5, 10, and 15 minutes), and then switched the laser to pulsed mode to collect a fluorescence lifetime data set for a shorter time window (5 minutes). The cw-exposed data can then be compared to the pulsed data at equivalent durations of laser exposure. A resulting lifetime trajectory is shown in Fig. 2b, and additional QD lifetime trajectories are shown in Fig. SI.4. The fluorescence lifetime is positively correlated with the fluorescence intensity, as expected,[30] and the distribution of fluorescence lifetimes is comparable for pulsed and cw excitation (Fig. 2c). The average and standard deviations of the fluorescence lifetimes (determined from single exponential fits as per Fig. 2a) in the ON state are 20 ± 4 ns and 22 ± 4 ns for pulsed and cw excitation, respectively. There is a slight shift of the pulsed distribution that favors shorter fluorescence lifetimes compared to the cw excitaiton. Above an average of 0.016 excitons per pulse, faster laser repetition rates have been reported to lead to shorter lifetimes due to the formation of charged excitons (*ie,* trions).[23] Under the current conditions, 2.7 excitons per pulse are produced, therefore, the lower fluorescence lifetimes observed with pulsed compared to cw excitaiton are likely the result of an increased probability of forming short-lived trions.

As illustrated in Fig. 3, histograms of $\Delta\tau_{fluor}$ values (the change in fluorescence lifetime from its initial ON state value) show significant differences between pulsed and cw excitation. The distribution of $\Delta\tau_{fluor}$ in cw-exposed QDs broadens by ~70% over a 15 min time scale, but shows no significant change in the center value (Fig. SI.5b). In comparison, the distribution of $\Delta\tau_{fluor}$ in pulsed laser-exposed QDs both broadens by 25% and its center value shifts to a fluorescence lifetime which is 6 ns smaller over a 15 min period (Fig. SI.5a), a ~30% decrease in τ_{fluor}. Given that photobleaching is common with pulsed excitation, it is likely that this change in fluorescence lifetime arises from an increase in the nonradiative recombination rate. This increase in nonradiative rate is not observed in cw-exposed QDs at the same average power. Control experiments in which the average exciton populations, rather than the average power, are kept constant exhibit similar shifts in the average fluorescence lifetime (Fig. 3d). Similar to the exciton-dependence of the blinking fall-off times, this commonality suggests that the decrease in fluorescence lifetime shares a common origin between pulsed and cw excitation and that photobleaching is very sensitive to the excitation power.

Comparisons of spectral diffusion and photon anti-bunching showed no influence of the type of excitation on these phenomena. Average single molecule fluorescence linewidths were 63 ± 8 meV and 59 ± 6 meV for the cw and pulsed excitation, respectively, with no significant changes over ~10 min time scales. The pulsed data exhibited a slightly larger range of peak shifts, an average shift of 7 ± 3 nm as compared to 4 ± 1 nm for cw, but neither showed a pronounced tendency to either blue or red shift. Single molecule spectra and a histogram of peak shift values ($\lambda - \lambda_0$) are included in the SI as Figure SI.6. Spectral diffusion is known to be closely correlated to blinking events

in both low[4] and ambient[31] temperature experiments, therefore, this similarity in spectral diffusion behavior is expected given the similarity in blinking behavior. Photon antibunching measurements, specifically the second-order emission intensity correlation function $g^{(2)}$, can be used to characterize the exciton and biexciton (BX) quantum yields (QYs).[28] In the case of cw excitation, the BX QY is related to the relative values of $g^{(2)}$ at time zero and at long time delays (*ie*, the height of the dip at $\tau = 0$ and $\tau \to \infty$); in the case of pulsed excitation, the BX QY is related to the relative areas of the time zero $g^{(2)}$ peaks and the satellite $g^{(2)}$ peaks. BX QYs were calculated for ~30 QDs that had been exposed to either pulsed or cw excitation and example $g^{(2)}$ measurements are shown in the SI Fig. SI.7. No significant differences were observed in the average BX QY between pulsed and cw excitation. Although the BX formation rate is an extensive property and very different between pulsed and cw excitation, the BX QY is itself an intensive property of the QD, therefore, this similarity is reasonably expected.

Conclusions

In summary, we have compared single QD fluorescence dynamics in an experiment in which all macroscopic variables are identical except for the nature of laser excitation. The most sensitive phenomenon to pulsed versus cw excitation is photobleaching. Compared to cw excitation, pulsed excitation results in a higher incidence of photobleaching, faster photobleaching rates, and decreases in the fluorescence lifetime upon extended laser exposure. Pulsed excitation also results in a lower probability of long ON states, as shown by the fall-off time values of 20 ± 2 s for cw compared to 44 ± 3 s for pulsed excitation. Spectral wandering, biexciton quantum

yields, and the power law exponents that describe fluorescence blinking are largely insensitive to the nature of laser excitation. These results suggest that direct comparisons of many important single QD phenomena between experiments using pulsed and cw excitation are fair even though exciton populations are very different. At the same time, caution regarding potential QD degradation, even at low average powers, is advisable when designing experiments with pulsed laser excitation.

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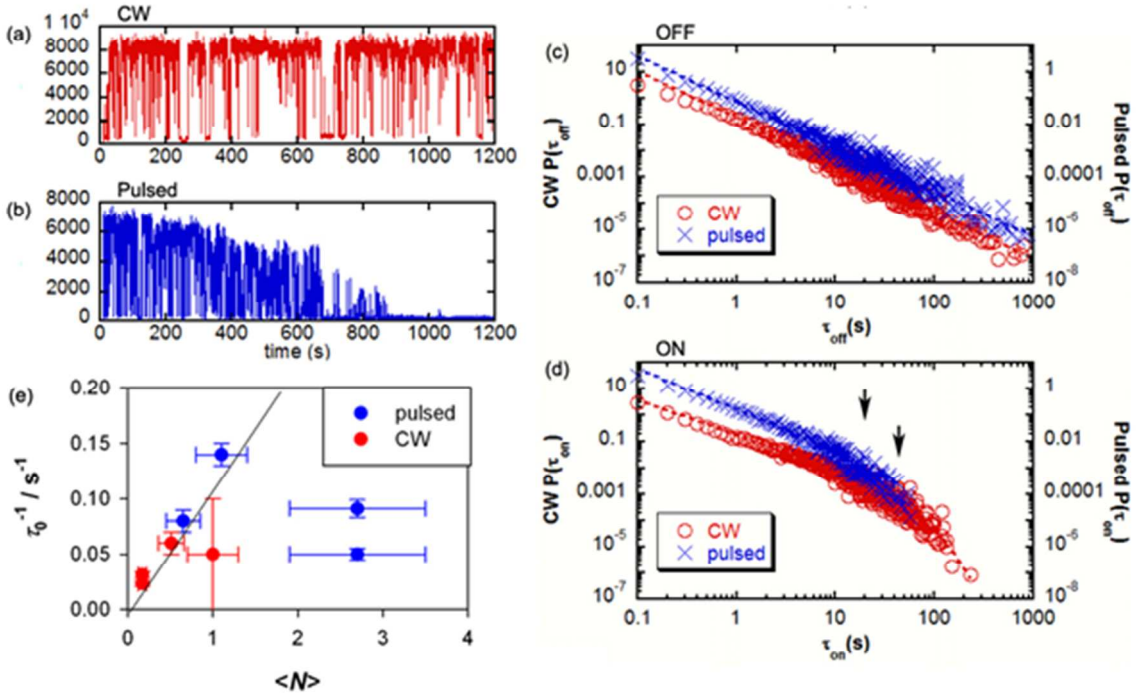

Figure 1. Representative single QD intensity traces are shown, with (a) cw and (b) pulsed excitation. Blinking statistics for the full data set are plotted on a logarithmic scale for both OFF (c) and ON (d) events. cw data is represented by red (O) scaled to the left y-axis and pulsed data is represented by blue (X) scaled to the right y-axis. Dashed lines are best fit lines as described in the text. Arrows in (d) indicate falloff values τ_0 of 20s and 44s for pulsed and cw, respectively. (e) The fall-off time dependence on exciton population for pulsed (blue circles) and cw (red circles) excitation. The solid line is a best fit line including all data points with $\langle N \rangle < 2$.

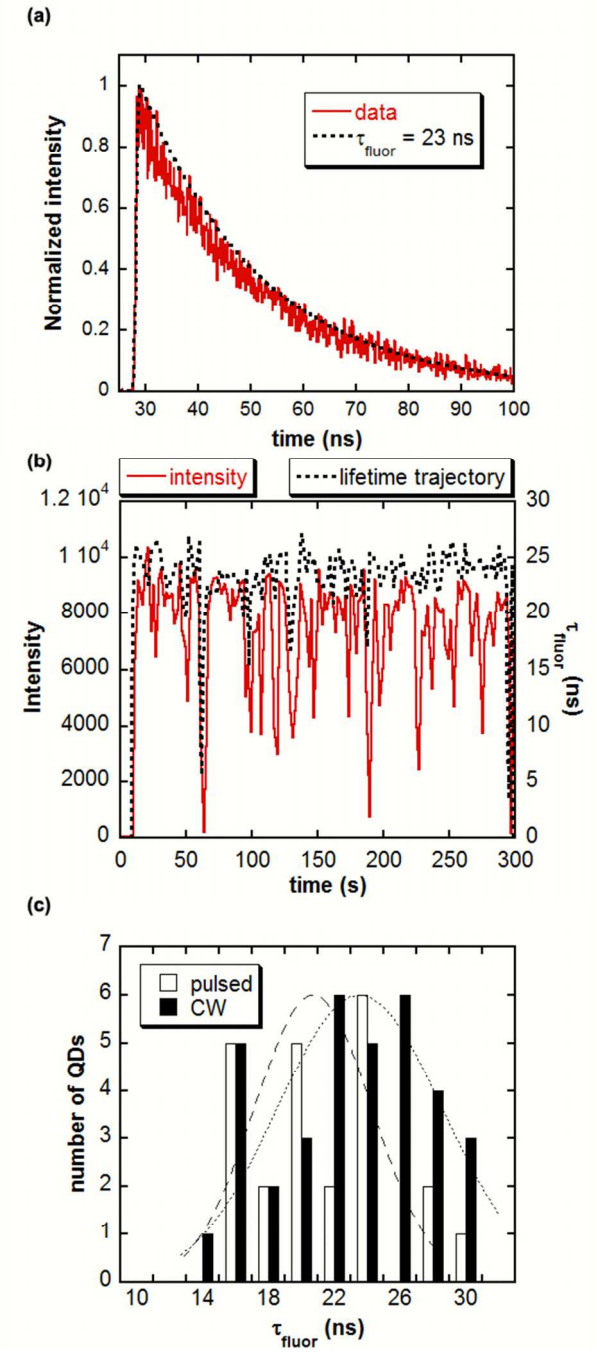

Figure 2. (a) Example fluorescence lifetime data from a single QD in an ON state (solid red line), and its best fit, single exponential curve (dashed black line). (b) A QD fluorescence intensity trajectory (solid red line, left y-axis) is overlaid with the calculated lifetime trajectory (dashed black line, right y-axis). (c) Histograms of the most probable fluorescence lifetimes in the ON state for pulsed (white bar) and cw (black bar) excitation. The distributions are fit to Gaussians lineshapes (dashed line for pulsed, dotted line for cw exposed).

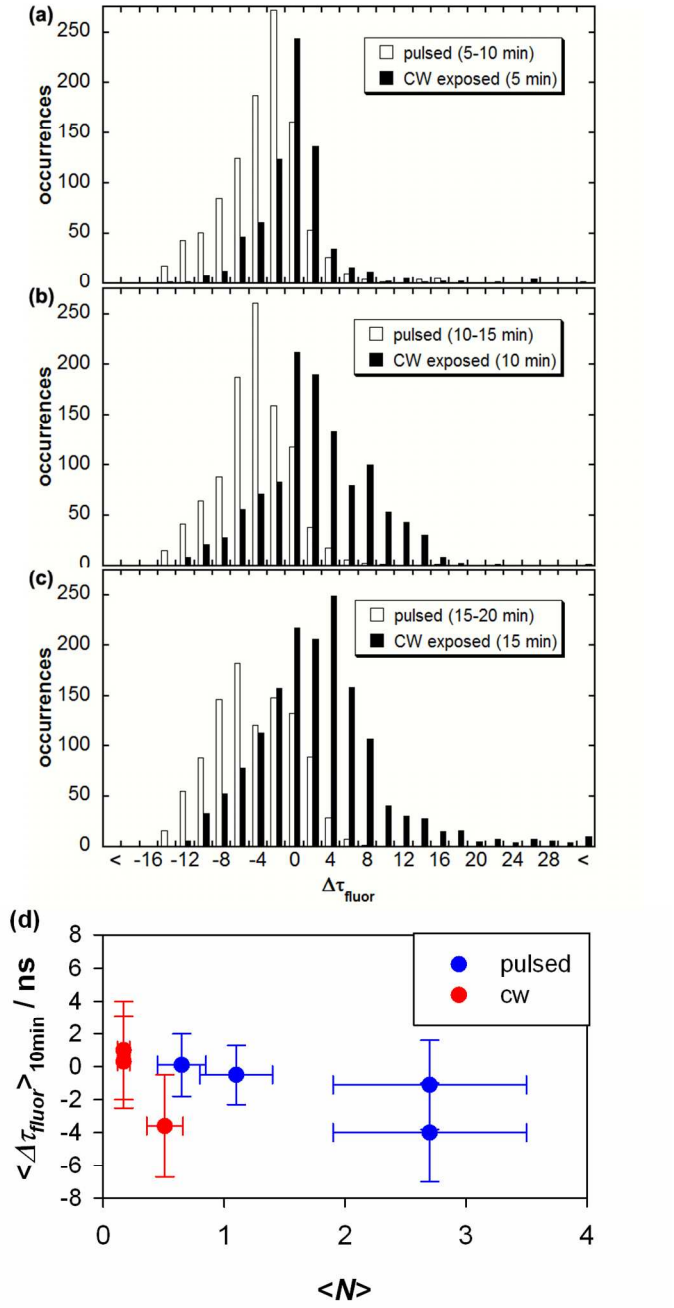

Figure 3. Histograms of the fluorescence lifetime deviations $\Delta\tau_{fluor}$ from 10 randomly selected QDs from pulsed (white bar) and cw (black bar) after (a) 5 min, (b) 10 min, and (c) 15 min of laser exposure. (d) The dependence of the average shift in fluorescence lifetime after 10 min of laser excitation on exciton population for pulsed (blue circles) and cw (red circles) excitation. Averages are determined from at least 8 QDs per data point and the error bars are 1σ standard deviations.

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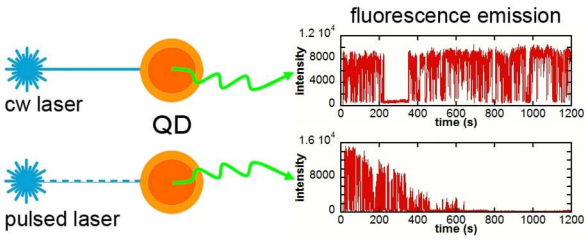

TOC Figure

Novelty Sentence:
Important similarities and differences between pulsed and continuous excitation on the fluorescence dynamics of single quantum dots were discovered.