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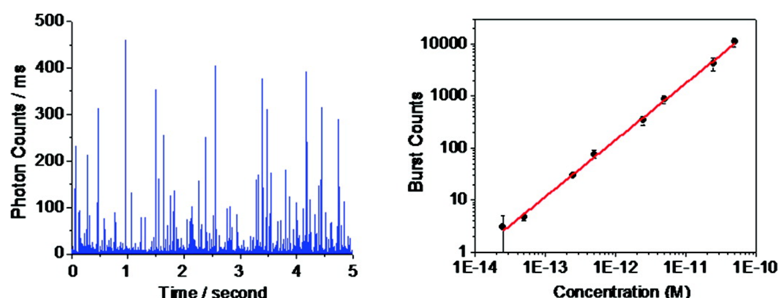

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Simple and Accurate Quantification of Quantum Dots via Single-Particle Counting

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Quantification of quantum dots (QDs) is essential to the quality control of QD synthesis, development of QD-based LEDs and lasers, functionalizing of QDs with biomolecules, and engineering of QDs for biological applications.[1−3] However, due to the composition- and size-dependence of QD absorption and fluorescence spectra,[1−7] and the change of absorption coefficient (ϵ) and quantum yield (Φ) values with pH, ionic strength, and surface coverage,[8,9] simple and accurate quantification of QD concentration in a variety of buffer solutions and in complex mixtures still remains a critical technological challenge. For example, the determination of ϵ value by a variety of techniques such as the gravimetric method,[4] elemental analysis,[5,6] osmotic method,[7] and controlled etching,[5] is often laborious, time-consuming, and somewhat inconsistent in the final results. Here, we introduce a new methodology for simple and accurate quantification of QDs via single-particle counting, which is conceptually different from established UV−vis absorption[4−7] and fluorescence spectrum[10,11] techniques where large amounts of purified QDs are needed and specific ϵ and Φ values are necessary for measurements (see Supporting Information, note). We demonstrate that single-particle counting allows us to nondiscriminately quantify QDs by their distinct fluorescence burst counts in a variety of buffer solutions and in complex mixtures regardless of their composition, structure, and surface modifications, and without the necessity of absorption coefficient and quantum yield values.

The principle of single-particle counting is based on single-molecule detection which can identify individual fluorescent molecules with a high signal-to-noise ratio.[12−14] The schematic for the experimental setup is shown in the Supporting Information, Figure S1. An argon laser was used as the excitation light source. The 488-nm beam was focused on the center of a 50-μm ID capillary by an oil immersion 100×/1.30 NA objective; the sample of QDs was moved through a laser-focused detection volume at a flow rate of 1.0 μL·min^{-1} by the pressure-driven flow from a syringe pump. Photons emitted from the QDs with a different emitting wavelength were separated by dichroic mirrors and detected by two avalanche photodiodes (APDs), respectively. Figure 1A shows the representative trace of fluorescence bursts from 605-nm-emitting streptavidin-coated CdSe/ZnS core−shell QDs (605QDs). A signal-to-noise ratio of up to 200 could be achieved under current experimental conditions, which allowed for the unambiguous distinguishing of QD fluorescence signals from background noise. Figure 1B shows the variance of burst counts as a function of QD concentration. Analysis of the burst counts (N) associated with QD concentration (C) yields equation $N = 10^{15.5} \times C^{1.1}$. This suggests that the QD concentration can be estimated from the measured burst counts; furthermore the QD concentration in the original solution can be obtained by multiplying C by the actual dilution factor. We tested other streptavidin-coated CdSe/ZnS core−shell QDs which emit at a wavelength of 700 nm (700QDs). An identical function

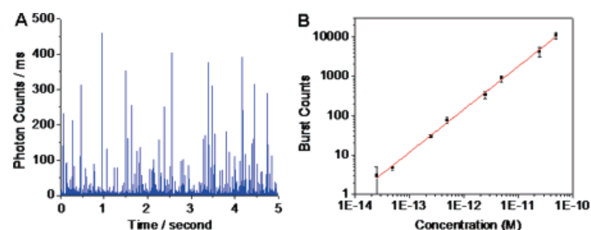

Figure 1. Principles of the single-particle counting for QD quantification. (A) Representative traces of fluorescence bursts from the 605QDs detected by single-particle counting. 605QD concentration: 2.5×10^{-11} M. (B) The variance of burst counts as a function of 605QD concentration.

between burst counts and QD concentration was obtained (see Supporting Information, Figure S2), suggesting that this single-particle counting method might provide a general approach for nondiscriminate quantification of QDs regardless of their composition and emitting wavelength, even without the necessity of accurate ϵ or Φ values. Additionally, the sample consumption of single-particle counting is at least 5 orders of magnitude less than that used in bulk UV−vis absorption or fluorescence spectrum measurement. Notably, this single-particle counting is much simpler and more straightforward compared with the method of analyzing the ensemble florescence intensity fluctuation.[15]

It should be noted that individual quantum dots exhibit fluorescence intermittency (i.e., blinking between on and off states) with time,[16,17] which is caused by a fluctuation in net charges inside or around the nanocrystal[16,18] and follows power laws.[17,18] As a consequence, only QDs in the on state can be detected.[17] To improve the accuracy of counting, we might add antiblinking agents such as β-mercaptoethanol,[19] mercaptothylanine,[20] propyl gallate,[21] and oligo phenylene vinylene ligand[22] to completely suppress the QD blinking; consequently all QDs passing through the focus volume can be efficiently detected.

Another important feature of this single-particle counting method is the ability to accurately assay QDs in a variety of buffer solutions, especially some of which might lower ϵ or Φ values, leading to an underestimation of QD concentration. For a proof-of-concept, we put the same amount of 605QDs in pure water and in acidic buffer, respectively. In the weak acidic buffer the 605QDs show decreased absorption spectra and decreased fluorescence spectra as well (see Supporting Information, Figure S3), which are indicative of lower ϵ and Φ values in the acidic buffer.[9,23] These results are in agreement with single-particle detection which reveals that fluorescence intensity of QD bursts significantly decreases in the acidic buffer (Figure 2B) compared with that in pure water (Figure 2A, note the difference in the scale of y-axis). However, the single-particle counting clearly demonstrated that the burst counts of QDs in acidic buffer were identical to those in pure water (Figure 2C), indicating no changes in QD concentration in spite of the decreased ϵ and Φ values in acidic buffer. This result shows that single-particle

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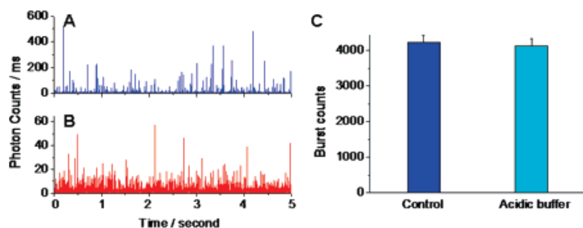

Figure 2. Quantification of QDs in a variety of buffer solutions via single-particle counting. (A and B) Representative traces of fluorescence bursts from the 605QDs in pure water (A) and acidic buffer (B) detected by single-particle counting. 605QD concentration: 2.5×10^{-11} M. (C) Comparison of the burst counts of 605QDs in pure water and acidic buffer detected by single-particle counting.

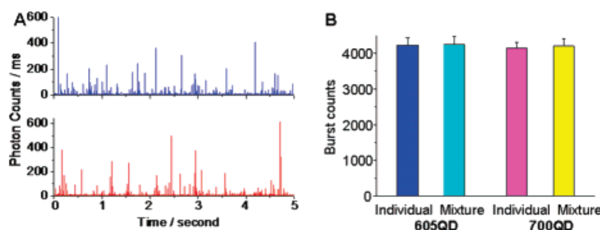

Figure 3. Simultaneous determination of multiple QDs in a mixture via single-particle counting. (A) Representative traces of fluorescence bursts from a mixture of 605QDs and 700QDs in the blue and red channels detected by single-particle counting. 605QD fluorescence signals are shown in blue; 700QD fluorescence signals are shown in red. (B) Comparison of the burst counts of 605QDs and 700QDs from the individual QDs and a mixture detected by single-particle counting. 605QD concentration: 2.5×10^{-11} M; 700QD concentration: 2.5×10^{-11} M.

counting provides a simple and efficient way to measure QD concentration in a variety of buffer solutions despite the variance of ϵ or Φ values with pH and ionic strength, even without the need of re-evaluating the modified ϵ or Φ values. It should be noted that this single-particle counting allows reliable measurement of QDs not only in aqueous solution but also in organic solvents, where QDs are often dispersed. It can also be used to detect the core-only (e.g., CdSe) QDs which emit approximately 10 times weaker than the core–shell (e.g., CdSe/ZnS) QDs.

The final important feature of this single-particle counting method is its ability to simultaneously determine the concentrations of multiple QDs in a mixture. To demonstrate this ability, the same concentration of 605QDs and 700QDs were assayed both individually and in a mixture, respectively. With only 605QDs present, only the 605QD fluorescence signals were detected in the blue channel; no 700QD fluorescence signals were detected in the red channel. With only 700QDs present, only the 700QD fluorescence signals were detected in the red channel; no 605QD fluorescence signals were detected in the blue channel (see Supporting Information, Figure S4). These results demonstrate that there is no cross-talk or spectral leaking between 605QD and 700QD emission spectra. Whereas in a 605QD/700QD mixture, both 605QD and 700QD fluorescence signals were simultaneously detected in the blue and red channels (Figure 3A). The burst counts of 605QDs obtained from the mixture were identical to those obtained from an individual 605QD solution (Figure 3B). Similar results were obtained for 700QDs which showed identical burst counts in both an individual 700QD solution and the mixture (Figure 3B). These results suggest that single-particle counting can unambiguously quantify individual QDs in a mixture, without the necessity of ϵ and Φ values. In contrast, there is no way to determine multiple QDs in a mixture using either UV–vis absorption or fluorescence spectrum measurement, because it is practically impossible to obtain accurate ϵ or Φ values for individual QDs from a mixture.[5,24]

In conclusion, a unique and general approach for the simple and accurate quantification of QDs has been developed via single-particle counting. This single-particle counting can non-discriminately quantify different kinds of QDs in a variety of buffer solutions regardless of their composition, structure and surface modifications, and without the necessity of ϵ and Φ values. It can also unambiguously quantify individual QDs in a complex mixture, which is practically impossible for both UV–vis absorption and fluorescence spectrum measurements. This single-particle counting is especially useful for the determination of unknown nanoparticle concentration where the normal assay is limited by the absence of ϵ and Φ values. Importantly, the application of this single-particle counting is not just limited to QDs but also can be extended to fluorescent microspheres, quantum dot-based microbeads, and fluorescent nanorods, some of which currently lack efficient quantification methods.

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Supporting Information Available: Experimental details; Note; Figures S1–S4. This material is avialbale free at http://pubs.acs.org.

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