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## Extracting Spectral Dynamics from Single Chromophores in Solution

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Fluorescence spectroscopy of single chromophores immobilized on a substrate has provided much fundamental insight, yet the spectral line shapes and dynamics of single chromophores freely diffusing in solution have remained difficult or impossible to measure with conventional linear spectroscopies. Here, we demonstrate an interferometric technique for extracting time dependent single chromophore spectral correlations from intensity correlations in the interference pattern of an ensemble fluorescence spectrum. We apply our technique to solutions of colloidal quantum dots and explore the spectrum of single particles on short time scales not feasible with conventional fluorescence measurements.

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Spectroscopic measurements on single chromophores can reveal rich complexity previously obscured by the averaging effects of sample and environmental heterogeneity. However, conventional single molecule fluorescence measurements are fundamentally limited in temporal resolution by the time necessary to accumulate a spectrum and require the chromophore to be both highly photostable and immobilized on a substrate. In this Letter, we demonstrate a method for extracting spectral correlations from single chromophores in solution. Our method uses continuous wave (cw) excitation, provides temporal resolution nearing the lifetime of the emitter, and reveals the evolution of the average single emitter spectral linewidth over nearly 6 orders of magnitude in time.

Fluorescence spectroscopy on single chromophores immobilized on a substrate has provided much insight into, for example, spectral diffusion of molecules [1] and nanoparticles [2], conformational dynamics of proteins [3], and temperature dependent line shapes [4]. However, there are times when stability, environmental dependence, or convenience make it necessary to measure spectroscopic characteristics of a sample in solution—even when single emitter information is desired. Powerful nonlinear techniques like photon echo and hole burning are available for solution phase measurements, but not all samples have an appreciable nonlinear cross section.

Our method fills the important niche of solution-based, single chromophore, cw spectroscopy by replacing the beam splitter in a standard fluorescence correlation spectroscopy (FCS) [5] experiment with a Michelson interferometer. We analyze intensity correlations as a function of interferometer position to obtain time dependent spectral correlations. Spectral correlations originating with the same chromophore are statistically enhanced and separable from the ensemble using intensity fluctuations from diffusion. Similar to the way FCS uses many particles diffusing through the focal volume to determine the average single particle diffusion coefficient, we use spectral correlations

from many diffusing chromophores to determine the average single chromophore spectral correlation.

We demonstrate the power of our technique using colloidal quantum dots (QDs). A single QD, like many molecules, experiences time dependent fluctuations in average emitted wavelength, causing the measured linewidth to be a time average of all relevant realizations [6]. Typical single QD fluorescence measurements at room temperature show a broad spectrum ( $\sim 40\text{--}70$  meV [7]), but it is not known if spectral dynamics are occurring during the time scale of the measurement (generally  $\geq 100$  ms). Furthermore, when QDs are synthesized, polydispersity in particle size creates a distribution of center wavelengths for spectra of individual particles, resulting in a broadening of the ensemble fluorescence spectrum.

Our experimental setup, shown in Fig. 1, and reminiscent of previous work done on photon correlation Fourier spectroscopy (PCFS) [8], consists of a homebuilt inverted microscope with a  $60\times$  water immersion objective (Nikon). The emission is spatially filtered through a confocal pinhole and sent to a Michelson interferometer, where the two outputs are detected with avalanche photodiodes (APDs, Perkin Elmer) and cross correlated with a hardware autocorrelator card (ALV-7004/Fast).

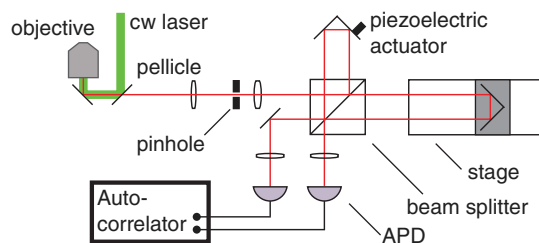


FIG. 1 (color online). The outputs of a scanning Michelson interferometer are cross correlated to convert fast frequency fluctuations into measurable intensity fluctuations. The sample is in solution and intensity fluctuations from diffusion allow for the separation of spectral fluctuations of single chromophores from the ensemble.

When observing an isolated single emitter with the setup, as in PCFS, the interferometer maps spectral changes into intensity changes and the distribution of spectral changes between photons with a given temporal separation determines the degree of correlation in the interferogram. By measuring the intensity correlation at different arm separations on the interferometer while dithering one mirror, a time dependent spectral correlation,  $p^{\text{single}}(\zeta, \tau) = \langle \int s(\omega, t)s(\omega + \zeta, t + \tau)d\omega \rangle$ , is obtained, where  $s(\omega, t)$  is the spectrum at time  $t$  and  $\langle \cdot \cdot \cdot \rangle$  denotes time averaging.  $p^{\text{single}}(\zeta, \tau)$  is then the probability of detecting two photons with a temporal spacing  $\tau$  and energy separation  $\zeta$ . From this, we learn about the temporal evolution of the emission line shape at time scales approaching the lifetime of the emitter [8]. PCFS has been demonstrated on single QDs immobilized on a substrate [9], but an object immobilized on a substrate does not necessarily behave identically in solution.

Here, we expand this interferometric technique to measure linewidths of single emitters obscured by an ensemble in solution and then demonstrate the extraction of spectral dynamics over a wide range of time scales. By combining the interferometer with FCS, we obtain a diffusion weighted spectral correlation,  $p^{\text{ens}}(\zeta) + [g^{\text{FCS}}(\tau) - 1]p^{\text{single}}(\zeta, \tau)$ , where  $g^{\text{FCS}}(\tau)$  is the intensity correlation measured in conventional FCS experiments and  $p^{\text{ens}}(\zeta)$  is the spectral correlation of the ensemble spectrum. The quantity  $p^{\text{single}}(\zeta, \tau)$  now coincides with the single emitter spectral correlation averaged over all observed emitters. Spectral correlations are independent of absolute energy so polydispersity in average emission frequency does not obscure spectral dynamics encoded in  $p^{\text{single}}(\zeta, \tau)$ . Below, we describe the successful extraction of  $p^{\text{single}}(\zeta, \tau)$  and refer readers to our previous simulations paper for more mathematical rigor [10].

For our initial experiment, we constructed the exceptionally polydisperse ensemble shown in Fig. 2(a) by mixing eight different sizes of CdSe QDs together in a solution of decane with trace Cd-oleate and decylamine. Each of the

single QDs in this ensemble is expected to have roughly the same spectral width despite vastly different center frequencies. The significant difference in linewidth for the single particle and ensemble spectra allows for a dramatic distinction between  $p^{\text{single}}(\zeta, \tau)$  and  $p^{\text{ens}}(\zeta)$ . After demonstrating the technique, we switch to a more uniform sample and extract detailed quantitative information about the spectral dynamics for a nearly monodisperse sample of QDs.

We begin by examining the emission of our artificially broadened ensemble at the outputs of the interferometer. When the path difference  $\delta$  between the arms of the interferometer is significantly longer than the coherence length of the spectrum, the intensity cross correlation is determined by the diffusion of the QDs, as seen in FCS. The FCS intensity correlation for our sample,  $g^{\text{FCS}}(\tau)$ , is shown in Fig. 2(b).

As the path difference  $\delta$  between the arms of the interferometer approaches the coherence length of the spectrum, interference influences the intensities at the two outputs of the interferometer to an extent determined by the Fourier transform of the spectrum. These intensities can be expressed as a sum over the intensities of all the individual  $N$  particles in solution, each with their own respective spectrum and interference pattern. The intensity cross correlation can then be written as follows:

$$g^{\times}(\delta, \tau) = \frac{\overline{I_a(t)I_b(t+\tau)}}{\overline{I_a(t)}\overline{I_b(t+\tau)}} = \frac{\sum_{i=1}^N \sum_{j=1}^N \overline{I_a^{(i)}(t)I_b^{(j)}(t+\tau)}}{\overline{I_a(t)}\overline{I_b(t+\tau)}}.$$

While measuring the cross correlation, we dither one mirror slightly to obtain the average cross correlation over a small distance. Dithering removes sensitivity to absolute energies while retaining information on relative energy separations. We can separate this cross correlation into parts dependent on pairs of photons emitted from the same QD ( $i = j$ ) and pairs of photons emitted from different QDs ( $i \neq j$ ). Only photons from the same QD have an intensity correlation that also depends on  $g^{\text{FCS}}(\tau)$  because

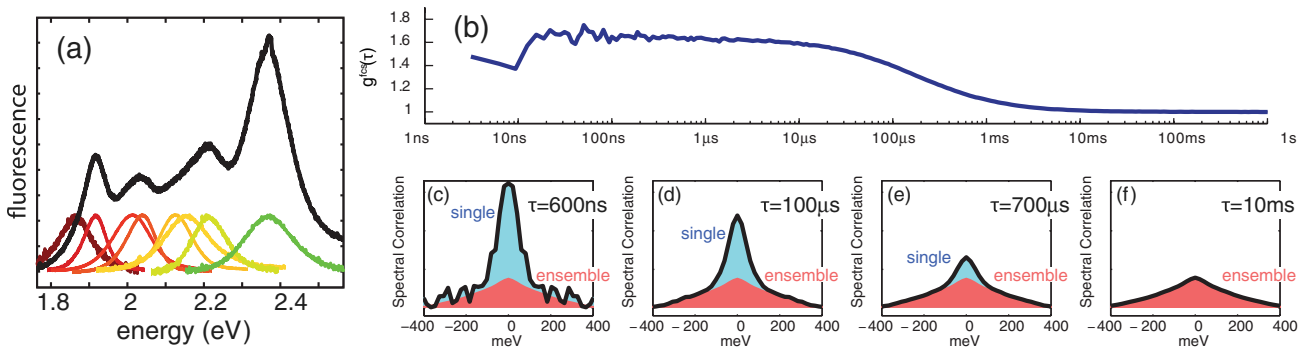


FIG. 2 (color online). (a) A polydisperse ensemble (black line) was constructed by mixing eight different colors of QDs to demonstrate the ability to extract the average single QD line shape. (b) The intensity correlation caused by diffusion,  $g^{\text{FCS}}(\tau)$ . (c)–(f) The diffusion weighted spectral correlation,  $p^{\text{ens}}(\zeta) + [g^{\text{FCS}}(\tau) - 1]p^{\text{single}}(\zeta, \tau)$ . As  $\tau$  increases, the single particle component decreases and, eventually, only ensemble information remains. For clarity, the time-invariant  $p^{\text{ens}}(\zeta)$  is superimposed on each figure. Each intensity cross correlation used was measured for 30 s with 457 nm excitation at 55  $\mu\text{W}$ .

the motion of different QDs is uncorrelated. This allows us to separate single QD spectral correlations from the ensemble. The number of QDs in our solution is very large ( $N \gg 1$ ) and the dithering rate is much slower than the time scales interrogated, so we simplify and obtain the following governing equation:

$$g^\times(\delta, \tau) = g^{\text{FCS}}(\tau) - \frac{1}{2} FT\{p^{\text{ens}}(\zeta)\} + [g^{\text{FCS}}(\tau) - 1]p^{\text{single}}(\zeta, \tau),$$

where  $p^{\text{single}}(\zeta, \tau)$  is the average single particle spectral correlation defined previously and  $p^{\text{ens}}(\zeta) = \langle S(\omega)S(\omega + \zeta) \rangle$  is the same for the ensemble with  $S(\omega) = \langle \sum s_i(\omega, t) \rangle$ . The cross correlation now depends on the Fourier transform (in path length  $\delta$ ) of the diffusion weighted spectral correlation,  $p^{\text{ens}}(\zeta) + [g^{\text{FCS}}(\tau) - 1]p^{\text{single}}(\zeta, \tau)$ . The diffusion weighted spectral correlation measured for our artificially broadened sample is shown in Figs. 2(c)–2(f). At short times, the enhanced probability of detecting photons from the same particle leads to a large single particle component in the spectral correlation. As the amplitude of  $g^{\text{FCS}}(\tau)$  decreases, the narrow  $p^{\text{single}}(\zeta, \tau)$  peak also decreases in magnitude while the broader  $p^{\text{ens}}(\zeta)$  remains constant. At the longest time scales,  $g^{\text{FCS}}(\tau)$  has fully decayed and only  $p^{\text{ens}}(\zeta)$  remains.

The static ensemble component can be removed from the diffusion weighted spectral correlation and we are left with only the time dependent spectral correlation of the average single particle. The remaining  $p^{\text{single}}(\zeta, \tau)$  fits to a Gaussian with FWHM of 104 meV at  $\tau = 5 \mu\text{s}$ . Assuming that the underlying spectrum is also Gaussian, we can extract an average single QD linewidth with a FWHM of 74 meV for this polydisperse ensemble. We are not aware of other experiments capable of extracting this linewidth at similar time scales and excitation conditions. Our result can be compared to linear measurements taken at longer time scales [7] and nonlinear measurements taken at higher powers [11]. The assumption of a Gaussian line shape leads us to a slightly larger value for the FWHM of the average single QD than these previous measurements. As demonstrated below, however, the spectral correlations we measure are nearly identical to the spectral correlations obtained with conventional single QD fluorescence spectroscopy.

In this first experiment, the count rate was  $\sim 15$  kHz on each detector. From fitting  $g^{\text{FCS}}(\tau)$ , we obtain the diffusion time for a QD moving through the focal volume to be  $\sim 0.18$  ms and the average number of QDs in the focal volume to be 1.5; thus, we are obtaining single particle spectral information with bursts averaging less than four photons from each QD traversing the focal volume. Assuming a 5% collection efficiency and an 80% quantum yield, the QD is excited, on average, every  $2 \mu\text{s}$ . This time scale is significantly longer than the temporal resolution of our technique, indicating that we have the ability to measure an intrinsic linewidth free of photoinduced dynamics from multiple excitation events.

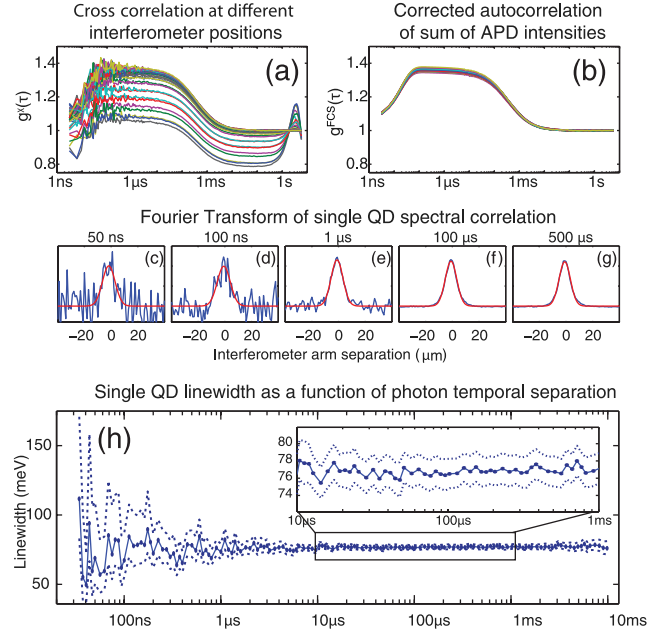


FIG. 3 (color online). Data on a nearly monodisperse sample of QDs emitting at 620 nm. (a) At each interferometer position a cross correlation is measured while slowly dithering one mirror. The sinusoidal intensity correlation from dithering appears only at long ( $> 70$  ms) times. (b) The autocorrelation of the sum of the two APD intensities is fit at short time scales to avoid effects from afterpulsing and dead times and then used as  $g^{\text{FCS}}(\tau)$ . (c)–(g) The extracted  $FT[p^{\text{single}}(\zeta, \tau)]$  with the Gaussian fits used in (h). (h) The linewidth (FWHM) for the average single QD is determined over nearly 6 orders of temporal magnitude by solving for  $FT[p^{\text{single}}(\zeta, \tau)]$  and assuming a Gaussian line shape. Dashed lines represent a 95% confidence interval. The spectrum is surprisingly constant over the temporal regime measured.

We now apply our technique to an ensemble of nearly monodisperse 620 nm CdSe/CdZnS QDs [12] illuminated with  $16 \mu\text{W}$  at 514 nm and spatially filtered through a slightly larger ( $50 \mu\text{m}$ ) pinhole. We acquire the cross correlation at a range of interferometer positions separated by  $1 \mu\text{m}$  and dither over  $\sim 1 \mu\text{m}$  distance [shown in Fig. 3(a)]. Each cross correlation is averaged for 40 s.

We simultaneously collect the autocorrelation of the sum of the two APD intensities,  $g^{A+B}(\tau)$ , which is fitted to avoid artifacts from short time scale afterpulsing and dead time and then used as  $g^{\text{FCS}}(\tau)$  in the governing equation. The fitted values replace the actual data only at short time scales ( $\tau \leq 6 \mu\text{s}$ ). For accurate fitting, we first measure  $g^{\text{FCS}}(\tau)$  with a cross correlation when the path difference  $\delta$  is large. We fit this to  $A[(1 - B * \tau^{2-m}) / (1 + \tau/\tau_D) - ke^{-\tau/n}]$ , which allows for diffusion, blinking, and antibunching [13] and adjust only its amplitude for each individual, afterpulse-corrected,  $g^{A+B}(\tau)$ . The afterpulse correction is determined from a count-rate-corrected removal of  $g^{A+B}(\tau) - g^{\text{FCS}}(\tau)$  as measured in a region beyond the coherence length [14]. The final intensity correlations used for  $g^{\text{FCS}}(\tau)$  are shown in Fig. 3(b).

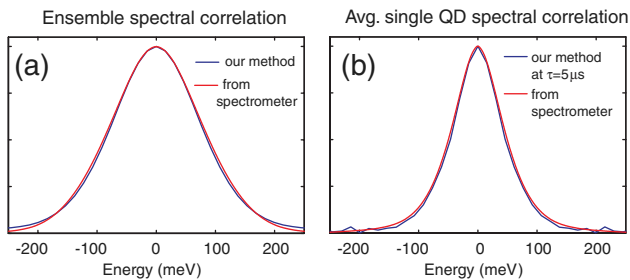


FIG. 4 (color online). Comparisons with conventional fluorescence spectroscopy. (a) The spectral correlation of the ensemble spectrum obtained with a spectrometer is nearly identical to that obtained by our method. (b) The single QD spectral correlation measured with our method (at  $5 \mu\text{s}$ ) is also nearly identical to the average spectral correlation of 25 single QDs measured with confocal microscopy and an integration period of 1 s.

For each  $\tau$  value between 34 ns and 10 ms we correct for  $g^{\text{FCS}}(\tau)$  and  $p^{\text{ens}}(\zeta)$  and extract the average single QD linewidth assuming a Gaussian line shape. Representative plots of  $FT[p^{\text{single}}(\zeta, \tau)]$  with Gaussian fits are shown in Figs. 3(c)–3(g). In Fig. 3(h), we plot the linewidth as a function of  $\tau$  and find it to be nearly constant over the temporal regime examined.

Spectral dynamics in QDs are thought to be caused by interactions with phonons on fast time scales and charging or discharging of surface states on slower time scales. While interactions with phonons are much too fast to be probed with this experiment, the slower, charging-based dynamics have previously been observed in single QDs at time scales from seconds to minutes. At cryogenic temperatures, the single QD linewidth becomes narrower with energy changes from spectral diffusion approaching that of the broader room temperature linewidth [15]. It has previously been unknown if these dynamics become faster at room temperature and become a significant contributor to the room temperature linewidth. Our result, however, demonstrates that the conventionally measured room temperature linewidth is not broadened by spectral dynamics at time scales between 34 ns and 10 ms and suggests that charging or discharging events, if they occur at all, must be on shorter or longer time scales.

As a control, we compare the  $p^{\text{ens}}(\zeta)$  from our measurement with a traditional ensemble spectrum measured through a spectrometer and then correlated with itself in Fig. 4(a). We find good agreement between the two techniques, further providing confidence in our ability to extract quantitative values from our method.

To compare our measurement with standard fluorescence measurements, we used confocal microscopy to measure the spectrum for 25 individual QDs and calculated the average spectral correlation using a 1 s integration time and  $440 \text{ W}/\text{cm}^2$  illumination from a 514 nm argon ion laser. This direct measurement, as compared to the spectral correlation obtained from solution at  $\tau = 5 \mu\text{s}$ , is shown in Fig. 4(b). The two spectral correlations are nearly iden-

tical, implying that the QD spectrum is not significantly influenced by the substrate.

In conclusion, we have demonstrated a method for extracting the average single emitter linewidth from intensity correlations in an ensemble fluorescence spectrum. By combining fluorescence correlation spectroscopy with an interferometer, we can separate single particle spectral correlations from the ensemble with a temporal resolution many orders of magnitude smaller than previous fluorescence experiments. Our method is relatively simple and can provide powerful information not easily discerned by other methods, especially when it is important to measure spectral properties in the native environment.

We applied our technique to determine the single QD linewidth and spectral dynamics from a solution of QDs. We find the single QD spectrum to be surprisingly static in the regions between 34 ns and 10 ms, with a spectral correlation width of 109 meV and spectral linewidth of 77 meV assuming a Gaussian line shape. We are able to extract this width and dynamics information despite significant polydispersity in the ensemble measurement.

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- [1] W. E. Moerner and M. Orrit, *Science* **283**, 1670 (1999).
- [2] S. A. Empedocles and M. G. Bawendi, *J. Phys. Chem. B* **103**, 1826 (1999).
- [3] T. Ha *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 893 (1999).
- [4] W. P. Ambrose, T. Basché, and W. E. Moerner, *J. Chem. Phys.* **95**, 7150 (1991).
- [5] D. Magde, E. L. Elson, and W. W. Webb, *Biopolymers* **13**, 29 (1974).
- [6] S. A. Empedocles, D. J. Norris, and M. G. Bawendi, *Phys. Rev. Lett.* **77**, 3873 (1996).
- [7] D. E. Gomez, J. van Embden, and P. Mulvaney, *Appl. Phys. Lett.* **88**, 154106 (2006).
- [8] X. Brokmann, M. Bawendi, L. Coolen, and J.-P. Hermier, *Opt. Express* **14**, 6333 (2006).
- [9] L. Coolen, X. Brokmann, P. Spinicelli, and J.-P. Hermier, *Phys. Rev. Lett.* **100**, 027403 (2008).
- [10] X. Brokmann, L. Marshall, and M. Bawendi, *Opt. Express* **17**, 4509 (2009).
- [11] M. R. Salvador, M. W. Graham, and G. D. Scholes, *J. Chem. Phys.* **125**, 184709 (2006).
- [12] P. Snee, Y. Chan, D. Nocera, and M. Bawendi, *Adv. Mater.* **17**, 1131 (2005).
- [13] S. Felekyan *et al.*, *Rev. Sci. Instrum.* **76**, 083104 (2005); R. Verberk and M. Orrit, *J. Chem. Phys.* **119**, 2214 (2003); B. R. Fisher, Ph.D. thesis, Massachusetts Institute of Technology, 2005.
- [14] M. Zhao *et al.*, *Appl. Opt.* **42**, 4031 (2003).
- [15] R. Neuhauser *et al.*, *Phys. Rev. Lett.* **85**, 3301 (2000).