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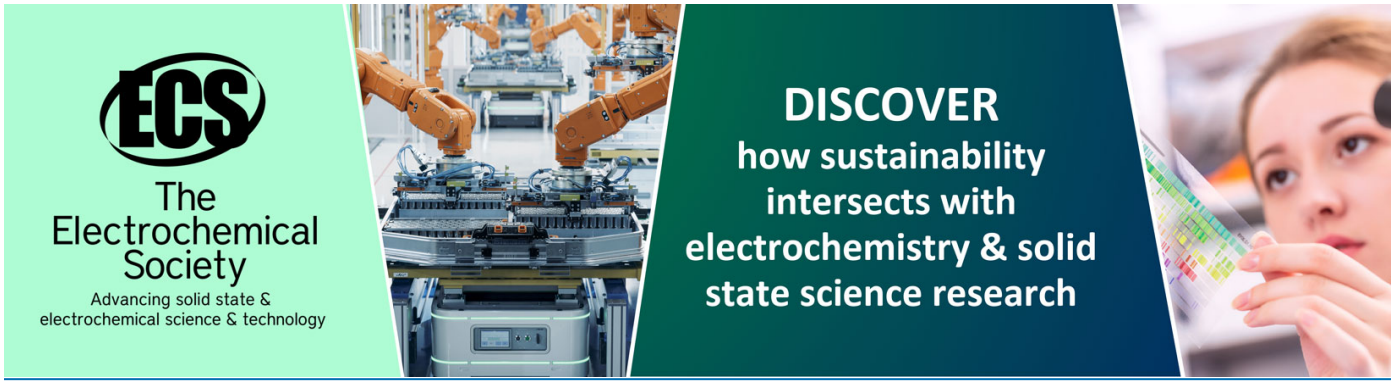

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Single Photon vs Two Photon Excitation of Silver Nanocluster

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Abstract. Silver nanodots, nanoparticles and nanoclusters have been of immense interest to researchers for some time now. A lot has been published in this field from synthesis to optical properties of various types of silver nanoclusters. In this work we explore the impact of polarized excitation on modulatability and fluorescence enhancement of silver (Ag) nanocluster. Ag nanoclusters as previously reported are enhancible and modulatable, however, for pathological application purposes emission in NIR even IR is desirable, so that the fluorescence signal detection over the auto-fluorescence background signal in vivo mode becomes easier. In a preliminary work we have seen a rise in anisotropic values beyond the maximum steady state anisotropy of 0.4 when the sample is excited using two colored laser light. Though there are numerous multi-photon study results available in literature, this is first of its kind where we use two polarized excitations to sequentially probe the excitable dark state of the cluster and the emission is then analyzed to obtain a value of steady state anisotropy along with changes observed in signal enhancement owing to modulation of the secondary excitation source.Ag nanocluster samples encapsulated by 10- or 12-mer ss-DNA capable of producing red to NIR light upon excitation at shorter wavelengths were prepared in-house.

1. Introduction

Beginning towards the end of 20th century there had been a lot of scientific research on metal nano particles and nanoclusters. Among several different kinds of metal nanoclusters studied Silver being cheap is used more widely. However, toxicity of Silver had always been a concern. An alternative approach using nanoclusters encapsulated in DNA have since been used following the pioneering work by Dickson research group at Georgia Tech [1]. Nanoclusters particularly the encapsulated ones have been shownto have wide application owing to their largely reduced toxicity and enhanced photostability. Successive studies have demonstrated that these metal nanoclusters can have wide range of photoemissivity and may exhibit enhanced fluorescence and become modulatable [2,3]. A range of encapsulated silver nano-dots and nanoclusters has been reported with photoemission power ranging from Visible to IR. Reportedly, the stability and fluorescence enhancement achieved from nanoclusters designed to emit in NIR-IR range is weak compared to those emitting in visible. However, these long wavelength emitting nanoclusters are preferred over the other ones emitting in the short wavelength regime for pathological application and biological imaging owing to their non overlapping with the auto fluorescence from biological samples. In this preliminary study, we probe changes in inherent anisotropy property of Ag nanoclusters encapsulated in ss-DNA capable of emitting red light up on excitation with NIR laser. The change in anisotropy of the nanocluster induced via use of two laser excitation may be further exploited for biological imaging purposes. Anisotropy of the sample in this study has been determined using two color excitations, usually a combination of Visible (green) and NIR lasers. Though there have been several reports on two photon anisotropy, however, those studies employ two photons coming from same initial source whereas in our method sequential absorption of two photons from two separate excitation sources are exploited, and the effect of individual excitation polarization on the total anisotropy has been examined.

2. Experimental Method

DNA encapsulated nanosclusters (AgNC) were synthesized using ss-DNA obtained from Integrated DNA Technologies (IDT) following the previously described method by Dickson research group [4,5].

The UV-Visible absorption spectrawere acquired using a Varian Cary and a Shimadzu UV-2401 spectrophotometer. The fluorescence emission spectra were acquired using Jobin Yvon Horiba Fluoromax-3 and Photon Technology International QuantaMasterspectrofluorimeter.The sample concentration was then readjusted to give adequate fluorescence photon counts when excited with a single laser (primary) excitation of 532 nm (PicoQuant diode laser). Few microliters of the sample was placed on a coverslip and the fluorescence emissions due to a single polarized excitation of 532 nm(PicoQuant diode laser operated at 10 kHzrepetition rate) were acquired using anAvalanche Photodiode Detector (APD).

Dual laser excitation spectroscopy was carried out using a 532 nm pulsed laser as the primary excitation and a 803 nm (NIR) continuous wave (CW) laser as the secondary excitation source. 532 nm shining on the sample though a 60X water objective prepares an initially excited state population that rapidly decays to lower energy states. As reported in several papers by Dickson research group, a part of the initial excitedpopulation decays to an intermediate dark state which in itself is not a fluorescing state but a second longer wavelength (secondary) excitation can put the population back in the fluorescing state and thereby enhancing the fluorescence yield from the sample.The CW NIR laser was used to excite the dark state population and the resulting increase in fluorescence was measured using a Perkin-Elmer APD and analyzed using a photon counting module (SPC-630, Becker Hickl and National Instruments board). At the beginning the plane of polarization of the two lasers were made parallel to each other. Co-illumination by the 532 nm and the NIR laser also resulted in an extended fluorescence lifetime estimated in microseconds. The fluorescence anisotropy transients were obtained using the acquired polarized fluorescence emission andthe maximum steady state anisotropy values were determined for primary only (Single photon anisotropy) and dual laser excitation (two photon anisotropy).

3. Results and Discussion

The fluorescence emissionfrom the AgNCwas split into two components using a PolarizingBeam Splitter prior to collection through two identical APDs. Theperpendicular and parallel polarized components were collected simultaneously. The fluorescence photon counts thus recorded were then combined to look at the signal enhancement upon co-illumination with two lasers,and the effect of excitation polarization on signal enhancement was also analyzed. The total fluorescence emission from the AgNC upon additional excitation with a secondary NIR laser was found to be higher than that obtained with a single primary only excitation of 532 nm. Fluorescence signal enhancement was found to be maximum when polarization of the two lasers are identical (parallel to each other). The anisotropy decay profile was obtained under different conditions using the polarized emissions along with the calculated G-factor for the set-up. Figure 1 shows example of anisotropy decays obtained for Ag-DNA cluster in water for one photon and two photon excitations. The maximum steady state anisotropy (r_0) was then obtained by fitting the experimental curve to an exponential decay model using MATLAB and Python programs. The r_0 value thus obtained for dual laser (two photon) excitation was found to be higher thanthat for a single laser (one photon) excitation. Considering the preliminary stage of this study drawing any conclusion on the variation of the r_0 or the correlation timescales will be far fetching. However, the relative polarizations of the two laser sources seemed to affect the resulting anisotropy decay which needs to be explored

further. We noted a change in the maximum (r_0) and the decay time upon a change in relative polarization of excitation source. While the maximum expected value of r_0 for single laser excitation was 0.4 for the dual laser excitation the maximum anisotropy $r_0$was estimated to be ~ 0.57 owing to an additional $\cos^2\theta$ dependence for the distribution of fluorophores in the sample. Interestingly, our findings though much in early stages suggest that r_0 could indeed be close to 0.57. We have consistently observed r_0 values > 0.4. Similar results had been reported for two photon anisotropy where the two photons were essentially similar [6,7]. However, in this study two photons of fundamentally different property were used. The preliminary results indicate effect of orthogonality of the excitation polarization on overall anisotropy of the nanoclusters and an in-depth analysis would be carried out to better understand the impact of relative polarization on anisotropy.

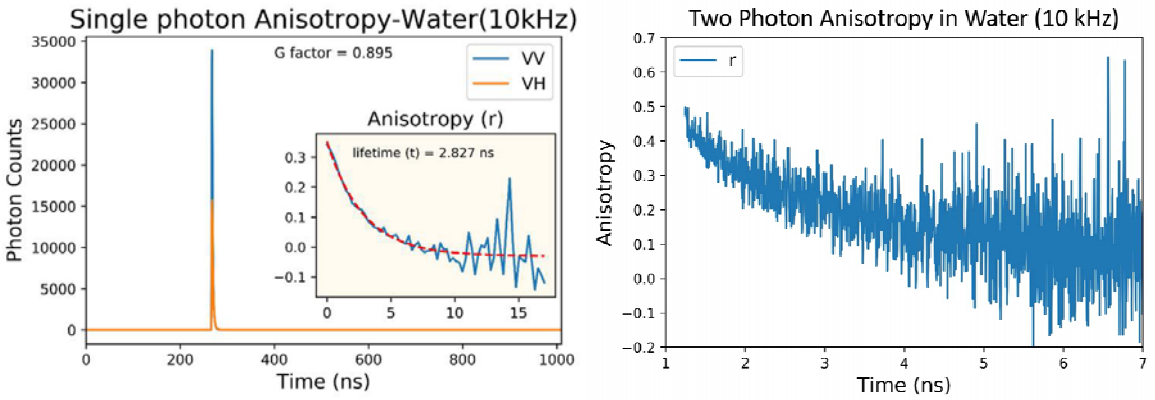

Figure 1 (Left) Polarized emission transients from AgNC encapsulated in DNA; VV representsparallellypolarized primary and secondary excitations, and VH represents primary excitation polarized perpendicular to secondary. The figure in the inset exhibit anisotropy decay as a function of time for primary only excitation of the cluster in water. (Right) Exhibits fluorescence anisotropy decay for the cluster in water upon dual laser excitation.

Though, the work is still much in its infancy we are consistently working at developing the experimental setup and, also streamlining the analysis process. We might be looking at signal deconvolution as a next step in order to subtract any photon contribution from other dark state induced effect following the second laser excitation in orderto better understand the current results.

4.Reference

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