

ENHANCED TWO POINT RESOLUTION IN FLUORESCENCE MICROSCOPY BY EXPLOITATION OF QUANTUM DOT BLINKING

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In microscopy, single fluorescence point sources can be localized with a precision several times better than the resolution limit of the microscope. Objects much smaller than the point spread function, such as quantum dots, which have a core size of approximately 5 nm, can be considered point sources. If within a group of sources, the emission light can be identified with a particular emitter, multiple sources can also be resolved and localized with high precision at distances less than the resolution limit. This has been previously demonstrated by separating particles by color, fluorescence lifetime, and bleaching.

We show that the intermittent fluorescence or 'blinking' of otherwise identical sources, such as seen in individual quantum dots, provides information about the individual sources that can be exploited to increase the two point resolution as compared to that achieved using only a single image with equivalent detected photons. Two methods of analysis are compared. First, using Independent Component Analysis to identify the light emitted by each source. Each emitter can then be localized individually. Second, a Maximum Likelihood Estimate on the time series, making no assumptions about the blinking model.

Although this technique has general application to any emitters having non-Gaussian temporal intensity distributions, which includes triplet state blinking, quantum dots are particularly suited for this technique since they are bright enough to image single nano-crystals with a typical epi-fluorescence microscope, are photo-stable, and have intermittent fluorescence on time scales as long as milliseconds and seconds.

We show that this technique is superior to a maximum likelihood based localization of the sum image assuming point emitters for both simulated and experimentally obtained data.