Fluorescence Intermittency Based Localization Microscopy for Nano-scale Imaging

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Short Abstract —**Fluorescence microscopy allows the study of cellular components with great specificity in live or minimally affected fixed cells. However, the ~ 200 nm resolution limit of the light microscope precludes studies of closely spaced components below this limit and many details of signal transduction are obscured. A method based on the intrinsic fluorescence intermittency of quantum dots is described that allows identification and localization of clustered membrane components well below the optical resolution limit with nanometer resolution. Results for fixed cell samples are shown and an extension for imaging dynamic behavior is described.**

Keywords — **Quantum Dot, Super-resolution, cellular membrane.**

I. Abstract

Accurate and meaningful modeling of cellular dynamics requires the use of data from real physical systems as either input or for comparisons between predictions and physical reality. Fluorescence microscopy is an essential tool for the measurement of static and dynamic distribution of cellular components. However, due to the wave nature of light, diffraction effects limit the resolution of the light microscope to roughly half the wavelength. Under typical conditions used for fluorescence imaging, the width at half maximum the Airy diffraction pattern of single point emitter is ~ 200 nm. Fluorophores distributed with inter-particle spacing less than this length become difficult to resolve due to the overlap of their observed intensity patterns. However, the position of single emitters that are spatially well separated from other emitters can be found with an accuracy better than 10 nm by fitting the observed intensity with the known diffraction pattern using the center coordinates as fit parameters [1]. New approaches have made use of temporal fluctuations in order to identify individual emitters within a cluster, leading to more accurate localization of each emitter [2-4]. Methods that rely on emitter intensity fluctuations, and that do not require the occurrence of isolated single emitters in a fluorescent state, have the potential of capturing dynamic processes. Another advantage of techniques that rely on the inherent temporal fluctuations of emitters under continuous illumination conditions is that this mode of operation is the most common in wide field fluorescence microscopes. This makes the technique immediately available for use by other researches with standard fluorescence microscopy setups with the addition of careful labeling conditions and appropriate analysis techniques.

Here we describe a Fluorescence Intermittency Based Localization technique specifically tailored to quantum dots (QDs) as the fluorescent reporters. The approximately two orders of magnitude larger excitation cross section as compared to visible fluorescent proteins and fluorescence dyes, as well as their photo-stability make QDs excellent candidates for single molecule studies. QDs undergo fluorescence intermittency under continuous illumination conditions (so-called "blinking) [5-7]. Generally, this phenomenon is undesirable in a fluorescent probe. However, when combined with the large excitation cross section and photo-stability, this property makes QDs excellent probes for localization microscopy studies.

We demonstrate a 'maximum a posteriori' (MAP) estimate [8] that makes use of measured QD characteristics to improve a maximum likelihood estimate of fluorophore positions. The technique is demonstrated with simulated data and with labeled membrane proteins in fixed cells. An extension for imaging dynamic membrane components is discussed.

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