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3D single molecule tracking and superresolution microscopy using multifocal plane microscopy

Sripad Ram, E. Sally Ward and Raimund J. Ober
Department of Immunology
University of Texas Southwestern Medical Center
Dallas, TX, USA.

Raimund J. Ober
Department of Electrical Engineering
University of Texas at Dallas
Richardson, TX, USA.
ober@utdallas.edu

Abstract- The study of cellular processes in three-dimensions is severely limited by the lack of imaging methodologies that allow for fast 3D tracking of cellular events and 3D superresolution imaging of sub-cellular structures. We have developed a 3D imaging modality, multifocal plane microscopy (MUM), that provides a powerful approach for 3D single molecule tracking and 3D superresolution microscopy. Here we review the technical challenges associated with 3D single molecule localization that is fundamental to both 3D tracking and 3D superresolution, and discuss how MUM overcomes these problems.

Keywords: Fisher information matrix, Cramer-Rao lower bound, resolution limits, 2D/3D localization accuracy, maximum likelihood estimator.

I. INTRODUCTION

Fluorescence microscopy represents a major tool for the study of intracellular trafficking processes in live cells. Recent advances have generated significant interest in studying the intracellular trafficking pathways at the single molecule level. Conventional microscopes exhibit poor depth discrimination especially near the focal plane of the objective ([3,7,9]), which results in high uncertainty in determining the z-location. This poses a fundamental problem for 3D single molecule tracking applications where high accuracy 3D localization of the single molecule is crucial. The same problem also arises in localization based superresolution imaging approaches such as (f)PALM and (d)STORM where the 3D location of the single molecule needs to be estimated with the best possible accuracy.

We have developed multifocal plane microscopy (MUM), which enables the simultaneous imaging of multifocal planes within a cellular specimen ([2,5,7,8,9,10,12]). MUM overcomes the longstanding problem of poor depth discrimination of conventional microscopes and enables high accuracy 3D localization of single molecules ([7,9]). In addition to this, MUM offers several advantages in that it can not only track multiple molecules at the same time over a large depth (~10 microns), but it also supports the 3D visualization of the cellular environment with which the single molecules interact. The latter is critical for deducing biologically relevant information.

II. 3D LOCALIZATION ACCURACY & MUM

An important question that arises in 3D single molecule tracking and localization based 3D superresolution imaging applications concerns with how accurately the position of the single molecule can be determined in 3D. To address this problem, we have made use of information-theoretic tools where we have calculated the Fisher information matrix ([16,17]) for the underlying parameter estimation problem of determining the 3D location of a point source from an optical microscope ([3,7,9,10,12]). Through the Cramer-Rao inequality ([17]), we obtain a limit to the accuracy (i.e., standard deviation) with which the 3D location can be determined. We have developed a rigorous stochastic framework to model the acquired data, where the photon detection process is modeled as a marked Poisson process. We also take into account deteriorating experimental factors such as pixelation, and additive Gaussian and additive Poisson noise sources ([1, 4]).

Our analysis shows that for a conventional microscope, the limit of the z-localization accuracy is worst when the single molecule is at the plane of focus ([3,7,9]), which makes 3D localization and in turn 3D tracking problematic with a conventional microscope. On the other hand, for a MUM setup, the limit of the z-localization accuracy is more or less a constant for a range of z-values including at the plane of focus ([3,7,9]). This enables high accuracy z-localization and in turn 3D tracking and localization based 3D superresolution imaging. It is important to note that the limit of the 3D localization accuracy for a MUM setup as well as for a conventional microscope exhibit inverse square root dependence on the number of detected photons.

By definition, the limit to the 3D localization accuracy provides the best possible accuracy with which the 3D location can be determined for a given experimental setting. Thus, a question arises as to whether there exists an unbiased estimator that attains this accuracy. We have demonstrated through simulations as well as experimental data that the maximum likelihood (ML) estimator of the 3D location attains the best possible accuracy ([9,10]).

The stochastic framework that was used to address the 3D localization problem provides a broad framework to study a wide variety of parameter estimation problems in optical microscopy. Using this framework, our group has carried out a

detailed analysis of the 2D localization accuracy problem ([1,4,10,11,14]) in optical microscopy. Further, we have shown that the ML estimator of 2D location attains the limit of the 2D localization accuracy in simulated ([1,4,11]) and experimental data ([10]).

III. 3D RESOLUTION & MUM

The resolution limit of an optical microscope plays a crucial role in determining the ability to measure the distance between two closely spaced point sources. Classical resolution criteria such as Rayleigh's criterion, although extensively used, are based on heuristic notions that render them inadequate for present day microscopy systems. Using the general stochastic framework that we have developed ([1,4]), we have addressed the issue of resolvability of closely spaced point sources for a conventional optical microscope ([6,13,15]) as well as for a MUM setup ([12]). We have derived analytical expressions for a new resolution measure that provide a limit to the accuracy with which the distance between two closely spaced point source can be determined in 2D ([6, 15]) and in 3D ([12,13]).

According to our new resolution measure, the resolution of an optical microscope is in fact not limited, and the resolvability is determined by the number of photons detected from the point sources. Through simulations and experimental data, we have validated the new resolution measure, where we have shown that distances as small as 10 nm can be accurately determined in a regular optical microscope with an accuracy predicted by the new resolution measure ([6, 15]).

We have carried a detailed investigation on the 3D resolution limits for MUM ([12]). We have also compared different experimental approaches that are currently used for measuring distances between single molecules ([12,15]). Our results show that MUM outperforms a conventional microscope in terms of 3D resolvability of single molecules ([12]) and is well suited for localization based 3D superresolution imaging.

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