Fast, simultaneous multiple-emitter fitting for single molecule super-resolution imaging

Fang Huang[1], Samantha L. Schwartz[2], Jason M. Byars[1] and Keith A. Lidke[1*]

Short Abstract — **Single molecule localization based super-resolution imaging techniques require repeated localization of many single emitters. We describe a method that uses the maximum likelihood estimator to localize multiple emitters simultaneously within a single, two-dimensional fitting sub region, yielding an order of magnitude improvement in the tolerance of the analysis routine with regards to the single-frame active emitter density. Multiple-emitter fitting enables the overall performance of single-molecule super-resolution to be improved in one or more of several metrics that result in higher single-frame density of localized active emitters. For speed, the algorithm is implemented on Graphics Processing Unit (GPU) architecture, resulting in analysis times on the order of minutes. We show the performance of multiple-emitter fitting as a function of the single-frame active emitter density. We describe the details of the algorithm that allow robust fitting, the details of the GPU implementation, and the other imaging processing steps required for the analysis of data sets.**

Keywords — **Super resolution, Single molecule localization, Maximum likelihood estimation, Fluorescence microscopy**

I. Introduction

Single molecule based super resolution techniques have revolutionized fluorescence microscopy, allowing spatial localization of single molecules to be determined with up to 20 nm resolution, an order of magnitude improvement from conventional fluorescence microscopy that is limited by diffraction to λ /2NA or approximately 250 nm [1,2]. The SM-SR concept relies on making precise and accurate estimations of the positions of individual emitters that label the biological structure. Conventional analysis approaches only attempt to localize well separated, single emitter events and data that does not fit this model is rejected.

We describe an analysis method that uses the Maximum Likelihood Estimator (MLE) in order to perform simultaneous position estimates of multiple emitters within a small sub-region. We proposed a robust algorithm which will be referred as Multi-emitter Fitting Analysis (MFA). A Chi-squared test statistics are proposed for model selection and a phenomenological modification are implemented on Fisher information matrix to deal with the singularity problem in Gaussian mixture models.

[1]Department of Physics, University of New Mexico, Albuquerque, New Mexico, 87131, USA

[2]Department of Pathology, University of New Mexico, Albuquerque, New Mexico, 87131, USA

*klidke@unm.edu

II. Image analysis

A. Image pre-processing

Raw images that are obtained from an EM-CCD camera are processed using a combination of uniform filter and maximum filter in order to obtain the positions of local maximum. Small sub regions are isolated around these local maximum and passed into MFA routine for single molecule analysis.

B. Multi-emitter Fitting Analysis (MFA)

Each sub-region is analyzed using MFA, which proceeds sequentially from an N = 1 model to an N = N_{max} model. The position estimates from the previous model is used to generate initial estimates for the current model in a deflation process which is demonstrated to be essential for fitting convergence.

C. Model selection and Uncertainty estimation

Based on log likelihood ratio test, we proposed a Chi-square distributed test statistic as an indicator of goodness of fitting. Trial model with the maximum p-value are then selected as a potential candidates for Super resolution image reconstruction. Uncertainty estimation for these position estimates are made using a modified Fisher information matrix which give correct result in both singular and non-singular affected regions.

III. Conclusion

The MFA method we have developed relaxes an important constraint on SM-SR technique which greatly increase the tolerance of single-frame density of active emitters. With MFA, since successful localization no longer requires isolated emitters, the performance can be improved in several metrics. For example: Data acquisition time can be reduced; A larger number of emitters can be localized; There is a higher tolerance on labeling density; and Dyes with higher minimum duty cycle can be used.

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