MAXIMUM-LIKELIHOOD BASED TRACKING OF FLUORESCENT NANOPARTICLES

François Aguet, Dimitri Van De Ville, and Michael Unser

Biomedical Imaging Group, Ecole Polytechnique Fédérale de Lausanne, Switzerland

INTRODUCTION: Novel fluorescent labeling techniques developed over the past decade have made it possible to study the dynamics of individual proteins inside living cells using fluorescence microscopy. These studies typically involve the acquisition of a time series of z-stacks, which are sequences of images taken at different focal distances. Single particle tracking techniques, designed to localize particles in three dimensions, have significantly contributed to the interpretation of such data. Due to the limited axial resolution of the z-stacks, it is common that particles must be localized from a single acquisition. As a consequence, since particles are likely to appear out of focus in the acquisition, determining their axial position is the most difficult aspect of the localization process. This is reflected in the literature, where many approaches to lateral localization (i.e., in the x-y plane) have been investigated (see, e.g., [1]), but only few methods for axial localization, with limited practical applicability, have been proposed.

METHODS: Fluorescent markers are usually much smaller than the optical resolution of a microscope and can therefore be assimilated to point sources. Accordingly, the image they produce at the output of the system corresponds to a slice of the microscope's three-dimensional point spread function (PSF). In this work we propose a new approach that exploits this property in order to retrieve the axial position of a particle.

To that aim, our image formation model combines a theoretical formulation of the microscope's PSF with a statistical model of the acquisition noise. An important consideration for this model is the fact that the PSF is non-stationary along the optical axis when the refractive index of the specimen is different from those of the immersion medium and coverslip, which in practice is almost always the case [2]. In our approach, we perform the localization by fitting this model to the acquisition(s) of a particle by means of a maximum-likelihood estimator for the axial position (see Fig. 1). To evaluate the performance of the estimation algorithm, we investigate the fundamental theoretical limits of this localization approach, and obtain a lower bound on the achievable localization precision, independently of the estimator used.

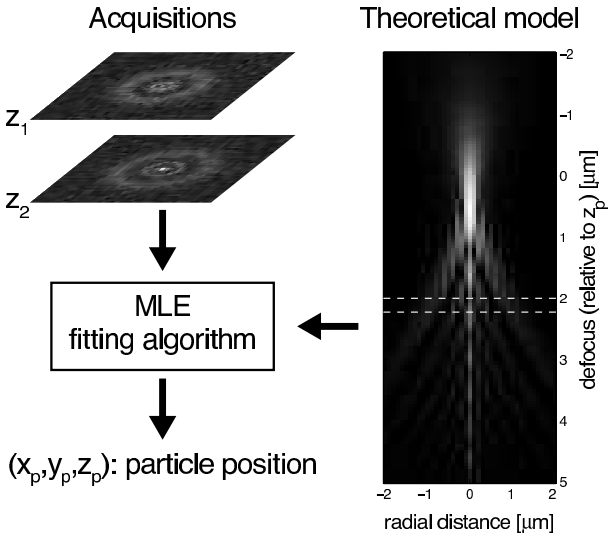

Fig. 1: Schematic representation of the fitting algorithm. In the example shown, two acquisitions of the particle are available.

We then extend the estimator to three dimensions, such that the complete positional information of particles can be recovered.

RESULTS: Using phantom acquisitions generated with our image formation model, we found that our estimator is optimal and reaches the fundamental limits discussed earlier. For a standard fluorescence setup, such as a 63×, 1.4 NA oil-immersion objective, these limits indicate that it is possible to determine the axial position of particles at the nanometer scale [3].

DISCUSSION & CONCLUSIONS: The results presented in this work show great promise for application in particle tracking studies. Preliminary experimental studies indicated that a precision close to the theoretically predicted value can be achieved. In addition, the theoretical investigation of the fundamental limits on precision leads to optimal acquisition settings that can be used as guidelines for experimentalists.

REFERENCES: [1]R.J. Ober, S. Ram, and S.Ward (2004) *Biophys J* 86:1185-1200. [2]S.F. Gibson and F. Lanni (1991) *J Opt Soc Am A* 8:1601-13.
[3]F. Aguet, D. Van De Ville, and M. Unser (2005) *Submitted.*

ACKNOWLEDGEMENTS: This work was supported in part by the Swiss National Science Foundation under Grant 200020-101821.