ON THE FUNDAMENTAL LIMITS OF NANO-PARTICLE TRACKING ALONG THE OPTICAL AXIS

F. Aguet, D. Van De Ville & M. Unser

Biomedical Imaging Group, EPFL, Lausanne, Switzerland

INTRODUCTION: Tracking fluorescent nano-particles is of particular interest for many applications in biology. Most tracking procedures try to reconstruct the particle's trajectory in an XY plane, or eventually in an XYZ volume when multiple acquisitions at different focal distances are available. In this work, we present the fundamental limits of recovering a nano-particle's position along the optical axis, from single or multiple plane z-stack acquisitions.

In microscopy imaging, nano-particles can be considered as point sources with respect to the microscope's resolution. Therefore, the observed image at a certain out-of-focus distance corresponds to the microscope's defocused point spread function (PSF). Given such an image, it is possible to determine the axial position of a defocused particle. In this work, we show the restrictions and limitations on the precision of this estimation procedure as a function of common imaging parameters and noise statistics. We also show how taking into account the influence of using multiple acquisitions at different focal distances can increase the precision.

METHODS: The Cramer-Rao Bound (CRB) gives the lower limit of the variance of the best possible unbiased estimator of the axial position. For a single image acquisition, we have previously shown that the precision depends on parameters such as the defocusing distance, the SNR and the resolution of the acquisitions [1]. Here we show how taking multiple images of the particle at different defocusing distances can further lower the CRB. By incorporating some general a priori knowledge about the particle's position (i.e. the range along the optical axis within which the particle is expected), the optimal positions of the acquisition slices can be obtained, yielding a remarkably higher precision. The precision achieved is of a few nanometers in optimal situations. For a single acquisition, the optimal position is at the center of the particle's interval. For multiple acquisitions, the focal distances are distributed symmetrically around the interval's center, but with non-trivial spacings.

RESULTS: Fig. 1 shows the CRB using a diffraction limited PSF model under the assumption of (signal-dependent) Poisson noise. The SNR of the in-focus image of the nano-particle is fixed at 20dB. Two examples of out-of-focus acquisitions at 1μm and 1.3μm respectively, are shown on the left (the effective SNRs are 9.16dB and 6.96dB resp.). The optimal bound shown in Fig. 2 is under the assumption that the particle follows a uniform distribution within the interval.

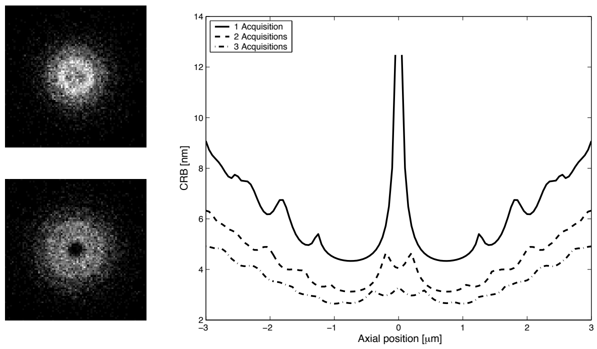

Fig. 1: Influence of multiple acquisitions on the CRB as a function of the particle's position along the optical axis.

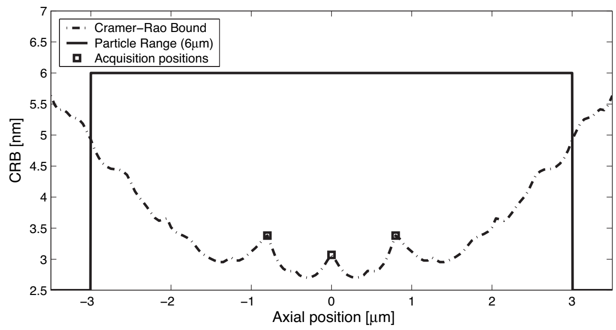

Fig. 2: CRB with three optimally spaced acquisitions (0.8μm between acquisitions).

DISCUSSION & CONCLUSIONS: Our theoretical findings can be used to deduce practical guidelines for optimal experimental acquisition settings in microscopy. In particular, time-lapse imaging can be combined with a piezo-electronic focusing device to iterate between the optimal acquisition positions. Our current work concentrates on estimating the location of nano-particles using a maximum likelihood-based approach.

REFERENCES: [1] N. Subotic, D. Van De Ville, M. Unser (2004) *On the feasibility of axial tracking of a fluorescent nano-particle using a defocusing model*. Second 2004 IEEE Int. Symp. On Biomedical Imaging, Arlington, USA.