THE EFFECT OF MOVEMENT ON THE LOCALIZATION PRECISION OF SINGLE MOLECULES AND PARTICLES

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In single particle tracking and single molecule imaging it is of crucial importance to determine the position of objects with a good precision. Therefore, a lot of effort has been put over the last few years in trying to estimate this precision. Recently, the exact expression of the localization precision for different intensity distributions and fitting algorithms has been published [1]. However, it was assumed that the object is stationary, which is not necessarily the case in a typical single particle tracking or single molecule imaging experiment. The movement of a particle during the observation time deforms the theoretically expected intensity distribution, which decreases the localization precision, as illustrated in Figure 1.

We extend the current localization precision formula to take the effect of movement during observation into account. We validate this extended expression by computer simulations and show that movement can deteriorate the localization precision significantly compared to the stationary case (up to a factor of 4 in Figure 1). However, determining the localization precision of moving particles experimentally is not straightforward, since the real position of the particle during the observation time is unknown and never the same. For this reason, to the best of our knowledge, this has never been measured experimentally before and stationary particles are typically used instead. Here we propose a new method that allows to calculate the localization precision of moving particles using the information from two different spectral channels. We validate this method by computer simulations and subsequently apply it to experimentally obtained single particle tracking movies. We show that the experimental results nicely correspond to the extended localization precision formula.

Figure 1: Simulated intensity distributions of a diffusing particle with diffusion coefficient 1 µm²/s for observation times (A) 10 ms, (B) 20 ms, (C) 30 ms.

Finally we demonstrate that the knowledge of the localization precision can be used to determine characteristics of diffusing particles from the mean squared displacement plot of a single particle tracking experiment more accurately. Furthermore, we expect that this work will be of relevance in determining the true resolution in localization based super-resolution microscopy methods like PALM and STORM in case the molecules are not fixed (e.g. in living cells).