

Accuracy of Localization Methods for Individual Fluorescent Probes

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Recent technological developments have made light microscopy of single molecules possible. The limited number of photons available from a single fluorescent molecule makes optimal data analysis as important to experimental resolution as improved experimental conditions, such as photo bleaching rates of fluorescent probes. The simple case of localization accuracy provides a pertinent example: A certain practice has developed for how to localize the center of the diffraction-limited spot that is the image of a fluorescent molecule (or bead, or quantum dot). It is easy to explain and apply, but expectations about its accuracy are confused, and its accuracy compared to what is theoretically possible, was unknown. We clear up the confusion and compare practice with Maximum Likelihood estimates that realize Fisher's information limit. The latter provides significantly enhanced accuracy, which decreases, however, with increasing background fluorescence. We test and demonstrate our analytical results on real experimental data.