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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 image analysis a statistical analysis. Consequently, optimal data analysis is as important to experimental resolution as improved experimental conditions, such as photobleaching rates of fluorescent probes. The simple case of *localization accuracy* provides a pertinent example. In theory, conventional lens-based light microscopy can determine the position of a point-like object with an accuracy that increases infinitely with the number of photons producing it. In practice, a finite signal-to-noise ratio limits localization accuracy and so may the choice of statistical estimator. Some estimators are easier to apply than others, but their relative virtues in regards to accuracy is unclear, or only known numerically for specific cases. We analyze three popular estimators under ideal conditions, find exact analytical results for their accuracy, and clear up a confusion in the literature. Next we test our results for accuracies against ideal real data, and find results that change our view of these estimators for practical use.