

# Fundamental performance limit for the resolution of a fluorescence microscope\*

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Rayleigh's resolution criterion for a fluorescence microscope is an important performance parameter for distinguishing two closely spaced point sources. Albeit its widespread use, it has long been recognized that this criterion is heuristic [1], and only provides an empirical measure of performance that does not consider the actual measurement process. For instance, Rayleigh's criterion neither reflects the stochastic nature of the photon detection process nor does it take into account detector characteristics, both of which influence the acquired data. Recent results from single molecule fluorescence experiments have shown that the location of two closely spaced point sources with distance of separation less than Rayleigh's criterion can be accurately measured (e.g., see [2]). These results suggest that Rayleigh's criterion may be inadequate as a performance parameter for the resolution of a fluorescence microscope. This then raises the question of what is the smallest distance of separation between two point sources that can be *accurately* measured with a fluorescence microscope?

By adopting an estimation-theoretic stochastic framework, we address the above question by deriving the Fisher information matrix [3] for the estimation problem of the distance of separation  $d$  between two point sources that are imaged by a fluorescence microscope. Through this approach, we derive a lower bound on the standard deviation of any reasonable (unbiased) estimator of the distance  $d$ . Since this lower bound is independent of specific estimation techniques [3], it provides a *fundamental performance limit* to determining the distance of separation between two closely spaced point sources. We illustrate our results to show how this performance limit governs the smallest distance of separation that can be accurately measured in a fluorescence microscope. Analytical expressions have also been derived that show how factors such as pixelation and noise sources affect the performance limit. The current approach provides a powerful tool to study various performance analysis related problems in fluorescence (optical) microscopes. Using this approach, we recently addressed a central problem in single molecule microscopy that is concerned with the accuracy with which the location of a single molecule can be determined. Here, we derived a simple analytical expression that provides a fundamental limit to the localization accuracy of a single molecule [4]. The present results have far reaching implications in single molecule fluorescence experiments, since resolution beyond Rayleigh's criterion in fluorescence microscopy opens up new opportunities to study nanoscale biological interactions especially within a cellular environment.

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