Title: Single-molecule and plasmon-enhanced fluorescence for super-resolution imaging of living bacteria

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Abstract: Single-molecule fluorescence brings the resolution of optical microscopy down to the nanometer scale, allowing us to unlock the mysteries of how biomolecules work together to achieve the complexity that is a cell. This high-resolution, non-destructive method for examining subcellular events has opened up an exciting new frontier: the study of macromolecular localization and dynamics in living cells. We have developed methods for single-molecule investigations of live bacterial cells, and have used these techniques to investigate three important prokaryotic systems: membrane-bound transcription activation in Vibrio cholerae, carbohydrate catabolism in Bacteroides thetaiotaomicron, and DNA mismatch repair in Bacillus subtilis. Each system presents unique challenges, and we will discuss the important methods developed for each system, in particular, a comparison of membrane-bound and soluble proteins, extensions to two-color and 3D imaging, and adaptations for studying live anaerobic cells.

Furthermore, we use the plasmon modes of bio-compatible metal nanoparticles to enhance the emissivity of single-molecule fluorophores. The resolution of single-molecule imaging in cells is generally limited to 20-40 nm, far worse than the 1.5-nm localization accuracies which have been attained in vitro. We therefore use plasmonics to improve the brightness and stability of single-molecule probes, and in particular fluorescent proteins, which are widely used for bio-imaging. We find that gold-coupled fluorophores demonstrate brighter, longer-lived emission, yielding an overall enhancement in total photons detected. Ultimately, this results in increased localization accuracy for single-molecule imaging. Furthermore, since fluorescence intensity is proportional to local electromagnetic field intensity, these changes in decay intensity and rate serve as a nm-scale read-out of the field intensity. Our work indicates that plasmonic substrates are uniquely advantageous for super-resolution imaging, and that plasmon-enhanced imaging is a promising technique for improving live cell single-molecule microscopy.

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