Abstract

Single-molecule fluorescence spectroscopy has revolutionized the field of biophysical sciences by enabling visualization of dynamic molecular interactions and nanoscopic features with high spatiotemporal resolution. Monitoring enzymatic reactions and studying diffusion dynamics of individual molecules (such as lipids and proteins) help us understand how these nanoscopic entities influence and control various biochemical processes. Nanophotonic antennas can efficiently localize electromagnetic radiation into nanoscale spatial dimensions comparable to single bio-molecules (<10 nm). These ultra-confined illumination hotspots thereby offer opportunity to follow single-molecule events at physiological expression levels.

In this thesis, we explore various photonic nanoantenna platforms (double nanohole apertures, dimer nanogap antennas and planar "antenna-in-box") and demonstrate their application in enhanced single-molecule fluorescence detection. Using fluorescence burst analysis, fluorescence correlation spectroscopy (FCS), time-correlated TCSPC measurements, and near field simulations, we quantify nanoantenna detection volumes, fluorescence enhancement factors and discuss the fluorescence photodynamic accelerations mediated by optical nanoantennas. An alternative to plasmonic structures, all-dielectric nanoantenna based on silicon nanogap is also demonstrated to enhance the fluorescence detection of single molecules diffusing in concentrated solutions.

Further, using resonant planar "antenna-in-box" devices we investigate the diffusion dynamics of phosphoethanolamine and sphingomyelin on the plasma membrane of living cells and discuss the results in the context of *lipid rafts*. Together with cholesterol depletion experiments, we provide evidence of cholesterol-induced nanodomain partitioning within less than 10 nm diameters and characteristic times being $\sim$100 μs.

Keywords: optical nanoantennas, fluorescence correlation spectroscopy (FCS), single-molecule detection, living cells