Playing With Lightning: Tip-Enhanced Fluorescence Microscopy at 10nm Resolution

Due to the limitations imposed by the diffraction limit, biological structure at the nanometer length scale is typically measured with non-optical forms of microscopy, such as electron microscopy (EM), X-ray diffraction, and scanning probe microscopy (AFM, STM, etc.). Although the spatial resolution of these techniques can be exquisite (~1 Angstrom for X-ray diffraction), they are either incompatible with physiological conditions or cannot differentiate between different biochemical species, or both. This restricts their application to studies of static phenomena or homogeneous systems, and excludes studies of dynamic interactions between various species of biomolecules. We developed an AFM-based optical microscope with spatial resolution that is comparable to EM, while adding the potential for time-resolved imaging of heterogeneous structure under physiological conditions. The microscope is based on a principle analogous to that of a lightning rod: the AFM probe concentrates the optical field within a focused laser beam. We measured an enhancement of up to 20-fold in the fluorescence rate from semiconductor nanocrystals (quantum dots), which was highly localized to within ~2 nm of the probe apex. We exploited the lightning-rod effect to generate optical images of the quantum dots with spatial resolution below 10 nm laterally and below 2 nm axially. I will describe the apparatus, our measurements, potential improvements, and applications for studying biological systems. One improvement in particular concerns the attachment of carbon single-wall nanotubes (SWNT) to the apex of AFM probes. The SWNT probes should increase the microscope resolution into the 1-2 nm regime and provide a platform for tethering single biomolecules, e.g. receptor ligands and enzymes, to achieve single-molecule biochemical precision. The combination of molecular-scale microscopy with single-molecule biochemical precision will enable a new class of biophysical experiments where specific biochemical sites can be addressed, and further, triggered to induce a particular reaction.