Apertureless near-field fluorescence microscope for biological imaging

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Abstract: We measured the optical response of fluorescent beads to sharp metallic and semiconducting probes, revealing several underlying near-field interactions. Our results suggest that ~10 nanometer optical resolution with spectroscopic chemical sensitivity is possible, and bear strongly on molecular-scale biological imaging.
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OCIS codes: (180.0180) Microscopy; (170.0170) Medical optics and biotechnology; (240.6680) Surface plasmons

Single molecule sensitivity at room temperature is now readily achieved using several fluorescence microscopy techniques. Fluorescence detection methods are particularly important to biological imaging at sub-cellular scales due to the high level of chemical differentiability provided by spectroscopic analysis. The spatial resolution of standard confocal and far-field optical techniques is limited, however, by diffraction to $\sim\lambda/2$, where λ is the wavelength, typically a few hundred nanometers. Aperture-type near-field optical probes have been used for fluorescence microscopy [1,2], but the resolution is typically limited to ~50 nanometers. Alternate microscopy techniques, such as electron microscopy (EM) and atomic force microscopy (AFM) are now routinely utilized to observe biological structure smaller than the visible light diffraction limit. Unfortunately, EM and AFM lack the high degree of chemical sensitivity characteristic to fluorescence microscopy and in the case of EM, dynamic measurement of a single system is not possible. At present, there are no microscopy techniques that simultaneously provide temporal resolution, molecular scale (<10 nm) spatial resolution, and chemical differentiability. We address this gap in technology by development of an AFM-based, apertureless near-field microscope designed for compatibility with aqueous environments and dynamic imaging of molecular-scale biological systems.

A schematic diagram of our instrument is shown in Fig. 1. It is based on a commercial tip-scanning AFM supported by a custom-built, laser-scanning inverted optical microscope with single-photon sensitivity. Our design incorporates the ability to correlate arrival times of individual photons with the oscillation phase of the vibrating cantilever. This phase correlation is used to measure modulation of the fluorescence rate as a function of tip position.

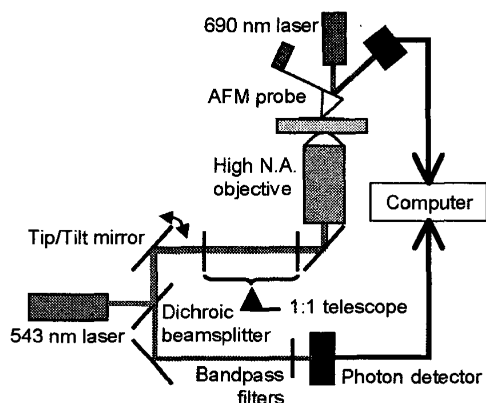

Fig. 1. Schematic diagram of apertureless microscope.

Three examples of measured approach curves using 20 nm diameter fluorescent beads are shown in Fig. 2. For metallic probes, fluorescent molecules within the bead can transfer energy non-radiatively into surface plasmon modes thereby reducing the amount of fluorescence radiated into the far-field detection region [1,2]. Additionally, the dipolar radiation pattern of each fluorescent molecule can be distorted towards the probe, again reducing the detected fluorescence [3]. For semiconductors, such as silicon, surface plasmons are not expected and no decrease

in fluorescence is observed. The fluorescence increase in this case is caused by localized probe-induced enhancement of the illumination field, described by electrostatic interaction between the probe and field [4].

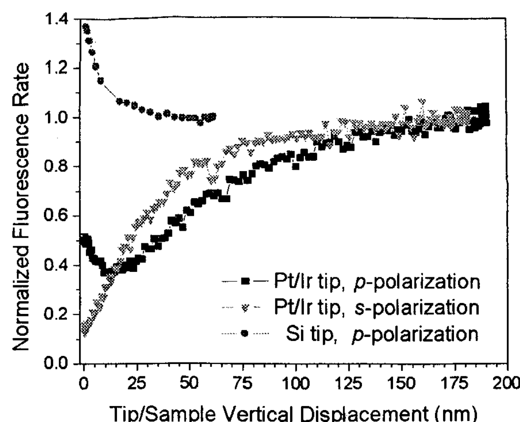

Fig. 2. Measured approach curves for 20 nm diameter fluorescent beads. Tip material and polarization direction are indicated in the legend. p-polarization designates an electric-field vector predominantly along the probe axis while s-polarization signifies an orthogonal electric field.

Electrostatic field enhancement only occurs when the illumination field vector is parallel to the probe axis [5]. We therefore employ a novel evanescent illumination scheme which allows for adjustment of the electric field vector along (p-polarization) or orthogonal to (s-polarization) the probe axis. We never observe increased florescence with s-polarized illumination, supporting the field enhancement interpretation. Further, when the silicon probe is allowed to dwell over the bead, the amount of increased fluorescence decreases more rapidly with time than does the background fluorescence. We attribute this to fast photochemical destruction of fluorescent molecules within a small volume of enhanced illumination field. Using Pt/Ir probes, partial recovery of strongly suppressed fluorescence occurs at very small tip/sample separations for p-polarization only. This also suggests the presence of field enhancement, and indicates a competition of enhancement with the aforementioned suppression mechanisms.

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