Nanoscale Fluorescence Microscopy Using Carbon Nanotubes

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Abstract - **We demonstrate the use of single-walled carbon nanotubes as nano-optical probes in apertureless near-field fluorescence microscopy. The carbon nanotubes strongly quench fluorescence leading to near-field contrast with spatial resolution of ~20 nm.**

INTRODUCTION

Tip-enhanced fluorescence microscopy (TEFM) combines the high resolution of atomic-force microscopy (AFM) and the chemical specificity of fluorescence microscopy [1-3]. In TEFM, the sharp probe of an AFM is positioned into the focus of a laser beam. The nano-optical field in the vicinity of the tip apex is strongly enhanced due to resonant excitation of localized surface plasmons or to the lightning-rod or antenna effects. Nanoscale resolution images are generated when the tip is scanned over the surface of a sample and the enhanced fluorescence signal is correlated with the lateral position of the probe. Since AFM can be used to characterize the topographical structure of protein complexes embedded in biological membranes *in vitro* [4], this technique has the potential to provide both structural and chemical information of membrane-bound bio-molecular networks [5]. Since single-walled carbon nanotubes (SWCNTs) have extremely small diameters (1-10 nm) and have favorable mechanical properties, they have been used extensively as AFM probes [6] and are attractive candidates for nano-optical probes. Here, we investigate the use of SWCNTs in TEFM and find that they provide strong near-field contrast and high resolution via quenching, rather than enhancement, of the fluorescence at the distal end of the tube.

NANOTUBE-PROBE FABRICATION

We grow carbon nanotubes on silicon substrates using chemical vapor deposition (CVD), and then attach them to commercial silicon AFM probes via the pick-up method [6]. In this technique, nanotubes are lifted off a CVD-grown substrate by an AFM tip via Van der Waals interactions as the tip is scanned over the nanotube-coated growth substrate. In general, the attached nanotubes are too long to be useful because of thermal vibrations at the distal end of the nanotube and because long tubes elastically buckle when loaded axially. Thus, the nanotubes usually must be shortened significantly, to a protrusion length of <100 nm. To shorten the attached SWCNTs, we apply electrical pulses between the nanotube and a conductive substrate to ablate carbon layers from the distal end of the nanotube. Controlling the voltage and duration of the pulses can alter the shortening steps. In this
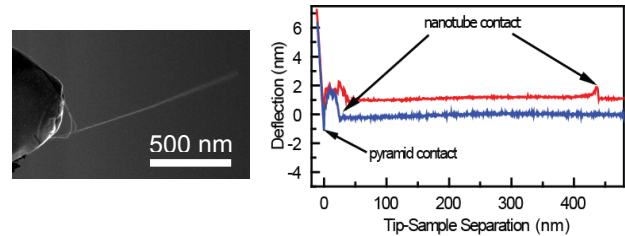

Fig. 1. Carbon nanotube probe. Left panel shows a field emission scanning electron micrograph of a SWCNT attached to a silicon AFM probe. Thermal vibration at the distal end of the nanotube can be seen in the image. Right panel shows AFM approach curves of a SWCNT-probe before shortening, (red curve), and after shortening (blue curve). There is a kink in AFM cantilever deflection when nanotube touches the surface, as indicated in the figure. The distance between these kinks and where the pyramid contacts give the length of the nanotube: ~440 nm before shortening and ~30 nm after shortening.

manner, many small voltage pulses can be applied until the nanotube reaches the desired length [6]. Figure 1 shows an electron micrograph of an attached nanotube before shortening as well as AFM force curves of a nanotube probe before and after shortening.

FLUORESCENCE IMAGING WITH NANOTUBE PROBES

Figure 2 shows tapping-mode AFM and fluorescence images of a CdSe-ZnS quantum dot (nominal size: 4 nm × 9.4 nm ellipsoid). Note that the topographical image of the quantum dot displays significant broadening and additionally the measured height is much smaller than the nominal diameter. Both of these effects are believed to be caused by the presence of a thin water layer on the sample surface under ambient conditions [7, 8]. Interestingly, these effects should be absent when the entire sample and tip are immersed under water. The fluorescence image shows a strong reduction in fluorescence signal, rather than enhancement, despite the fact that the experimental conditions are identical to those that give ~20-fold enhancement in the fluorescence signal from quantum dots when bare silicon tips (i.e. no SWCNTs) are employed [1]. We have made similar measurements on several hundred fluorescent particles, both dye-doped latex spheres and CdSe-ZnS quantum dots, using more than 50 different nanotube probes. In each case, enhancement is consistently absent.

It is well known that metal surfaces and particles can quench fluorescence from nearby fluorophores via non-radiative processes that dissipate the transferred energy via ohmic losses in the metal. However, we expect the nanotube

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growth and attachment procedure described above to yield a mixture of metallic and semi-conducting nanotubes. While it is somewhat surprising that all nanotubes tested exhibit strong quenching behavior apparently independent of whether they are semiconducting or metallic, they nonetheless generate strong near-field contrast with resolution of ~20 nm.

We can obtain additional information about the length scale of fluorescence quenching by measuring approach curves whereby the lateral scanning motion of the AFM cantilever is halted above a quantum dot and the nanotube is then precisely lowered onto the dot from a particular height. We have measured many such approach curves using more than 10 different nanotube tips on many different quantum dots. In all cases, the quenching starts 15-25 nm away from the quantum dot, as shown in Fig. 3. Again, the point at which the nanotube touches the quantum dot is indicated by the kink in the cantilever deflection signal.

In Fig. 2, the peak-to-peak oscillation amplitude of the probe was ~24 nm, slightly larger than the quenching interaction length shown in Fig. 3. Thus, we expect that the distal end of the nanotube spends a significant portion of its oscillation cycle outside the region of strong quenching. This should reduce the contrast as the fluorescence rate is averaged over the entire oscillation cycle. However, in Fig. 2, we observe essentially complete quenching over the area of the quantum dot. This may be an indication that quantum dots need some time to recover following a quenching interaction. This could be the result of charge transfer between the quantum dot and nanotube since the diffusion of excitonic electrons into the ZnS shell is known to cause a reduction in nanocrystal quantum efficiency [9]. The oscillation frequency of the AFM probe in this case is ~75 kHz, so the recovery timescale would have to be longer than ~10 μs. We will investigate this issue in the future using time-resolved measurements. If charge transfer is important in this case, it has strong implications for the potential use of nanotube-nanocrystal composites in photovoltaic and photosensor applications.

Fig. 3. AFM approach curve on top of a quantum dot using a SWCNT-tip. Blue curve is the AFM-cantilever deflection, where the small "kink" indicates where the CNT touches the surface. The fluorescence signal was recorded simultaneously, as shown in red.

nanotubes tested (>50) produced strong fluorescence quenching, despite the fact that we expect a mixture of metallic and semiconducting nanotube tips. The difference in behavior between silicon AFM tips and nanotubes may be a consequence of the one-dimensional nature of charge and energy transport in nanotubes. We plan to explore this issue through careful spatial measurements of the quenching efficiency using pulsed lasers to measure the lifetime directly. Further, we will explore more robust nanotube attachment methods so that the SWCNT-probes can be used in water to probe biomolecular networks.

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CONCLUSIONS AND FUTURE DIRECTIONS

We have demonstrated that carbon nanotubes can be used for nanometer-scale imaging of fluorescent samples. All
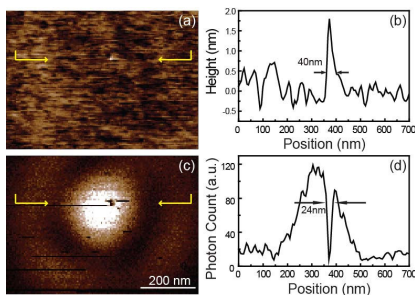

Fig. 2. (a) AFM topography and (c) fluorescence images and corresponding cross sections (b) and (d) of a CdSe-ZnS quantum dot using a CNT-probe. A reduced height of 2 nm for the quantum dot was observed as shown in (b). An optical 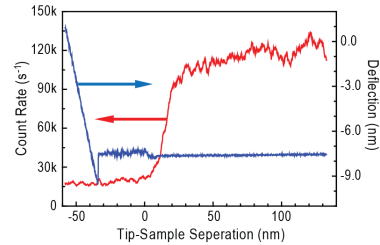resolution of 24 nm was achieved as indicated in (d).