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Carbon Nanotubes as AFM Tips: Measuring DNA Molecules at the Liquid/Solid Interface

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High-resolution imaging of soft and weakly adsorbed biomolecules on surfaces within buffer solutions presents a big challenge in research. Here we demonstrate that a single carbon nanotube could be used as a tip for probing biomolecules in solution using atomic force microscopy (AFM). Combining the unique properties of carbon nanotube tips with the newly developed magnetically driven oscillating AFM technique, we were able to obtain molecular-level, high-resolution images of DNA molecules adsorbed on the mica surface in solution. The length and the buckling force of the single nanotube tip can be derived directly from the force–distance curve. These measurements indicate that the nanotube tip is quite resilient, and it can quickly recover to its full length after repeatedly being crashed on the surface with excess force. The well-defined tip geometry provides extraordinary resolution in AFM imaging. The application of carbon nanotubes as AFM tips could lead to breakthroughs in biological studies.

KEYWORDS: carbon nanotube; AFM; molecular images; DNA

INTRODUCTION

Atomic force microscopy (AFM) is a powerful technique in revealing the microscopic structure of a variety of materials down to atomic level. It has become an essential technique for surface characterizations. However, there are still some long-standing problems in obtaining consistent high-resolution images. The radius and shape of conventional AFM tips vary from one to the other, and typically have tip radii of 10–50 nm. The dimension of the tip, in many cases, limits the resolution that AFM can achieve. In addition, AFM tips are often worn down quickly during measurements, resulting in the loss of lateral resolution. Recently, it has been demonstrated that multiwalled carbon nanotubes (MWNTs) attached to tapping mode AFM tips exhibit advanced imaging capabilities for measurements in air.[1–3] Because of their sharp geometry, intrinsic electrical conductivity and mechanical resilience, carbon nanotubes could be an ideal solution for the long-standing tip problem for AFM.

Because the nanotube tip presents a well-defined atomic configuration at the very end of the tip, it also provides us with a nice model system to study the tip–sample interactions. In addition, current studies of single biomolecular forces between sample surfaces and modified AFM tips face the difficulty of unknown tip geometry and thus an unknown number of molecules involved. The single-molecule bond-rupture forces are typically derived using mathematical models and assuming a round tip with a certain radius.[4–6] With nanotube tips, particularly close-domed tips, it is possible to modify only the very end of the tip with biomolecules in a well-controlled way[7] and thus the single molecular interaction can be measured directly from the force–distance curve. It is thus of great interest in developing nanotube tips for measuring biological samples, particularly within buffer solutions. We demonstrate the method for implementing and characterizing carbon nanotubes as AFM tips for measuring DNA molecules at the liquid/solid interface. This is the first report on using carbon nanotubes as AFM probes to image biomolecules within controlled buffer solutions at molecular resolution.

EXPERIMENTAL RESULTS

Previous studies on using carbon nanotubes as AFM tips were carried out with tapping mode AFM (Digital Instruments, Santa Barbara) in air.[1–3] However, we found that the nanotube was unstable using conventional liquid imaging cells, and we were unable to obtain images. This is likely to be caused by the disturbance to the tip by the surrounding fluids because a large acoustic vibration had to be applied to generate the AFM cantilever oscillation during the measurement. To use carbon nanotube as AFM tips for measuring soft biological samples in buffer solutions, the disturbance to the tip has to be minimized. Han *et al.*[8] recently reported that a magnetically driven oscillating probe microscope can be operated with a much lower amplitude (a few nanometres) in fluids. The disturbance by the surrounding fluids was also minimized because the oscillating force was applied directly to the cantilever. Images of biomolecules in fluids have been reported with the highest resolution using this technique.[8–10] We have adopted magnetically driven

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oscillating probe AFM (MAC Mode, Molecular Imaging, Phoenix, AZ) in this study.

A PicoSPM (Molecular Imaging, Phoenix, AZ) controlled with a MAC Mode module (Molecular Imaging, Phoenix, AZ) and a Nanoscope IIIa controller (Digital Instruments, Santa Barbara, CA) were utilized. Figure 1 shows a schematic diagram of the cantilever with carbon nanotubes attached to it as the probe. We used a silicon nitride cantilever with a spring constant of $k = 0.1$ N m^{-1} (Microlevers, Park Scientific Instruments, Sunnyvale, CA). The back of the cantilever was coated with a proprietary magnetic material and magnetized along the flexible axis of the cantilever by Molecular Imaging Co. (Phoenix, AZ). After coating, the spring constant of the cantilever only increases slightly (<20%).[8] The cantilever was driven at a frequency of ~30 kHz by a solenoid under the sample plate during the measurement. The a.c. oscillation amplitude and d.c. deflection of the cantilever were input into separate channels. A bundle of MWNTs was attached to the pyramidal tip of the cantilever with an acrylic adhesive as reported previously.[1–3] To strengthen the attachment, some tips were sputter coated with a chromium film after the nanotube bundles were attached. The diameter of the bundles ranged from tens to hundreds of nanometres. Typically, a single MWNT extended out of the bundle. Its length was controlled by a discharge method.[1] This single MWNT was then used as a probe for AFM imaging.

During AFM measurements, the cantilever and the tip were submerged within the buffer solution, which was sealed in a 0.25 ml Teflon cell on a piece of freshly cleaved mica. The DNA molecules were spontaneously bound to the mica surface from 2 μg ml^{-1} DNA solutions (lambda DNA, Hind III, New England BioLabs) and ~1 mM $MgCl_2$ was added into the buffer solution to enhance the interaction between DNA molecules and the mica substrate.

In previous reports,[1,2] the carbon nanotube AFM tips were inspected under scanning electron microscopes and transmission electron microscopes. In this study, we found that the single carbon nanotube tip presents quite characteristic force–distance curves, which can be used to derive directly the length and buckling force of the single nanotube at the very end of the tip. Figure 2 shows the oscillation amplitude and the deflection of the cantilever recorded simultaneously vs. the tip–sample distance (Z). Both curves were measured during tip approach towards the sample surface. The deflection of the cantilever was converted to the force between the tip and the surface by the linear relation $F = kD$, where $k \sim 0.10$ N m^{-1} is the spring constant of the cantilever and D is the deflection of the end of the cantilever (in nm). The vertical lines in Fig. 2 correspond to where fundamental changes occurred, with $Z_0 = 34.4$ nm, $Z_1 = 0$, $Z_2 = -56.5$ nm, $Z_3 = -311.8$ nm and $Z_4 = -333.9$ nm, respectively. These two curves are defined by a MAC Mode AFM mechanism and single carbon nanotube mechanics.

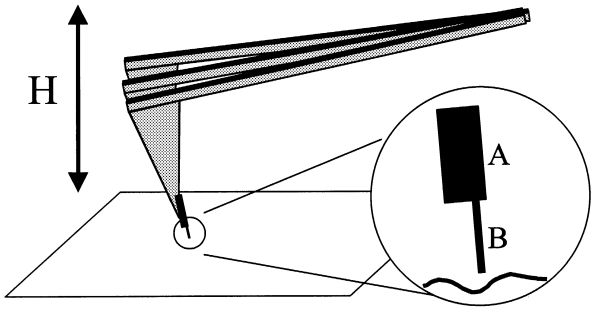

Figure 1. A schematic of the MAC Mode cantilever and carbon nanotube tips.

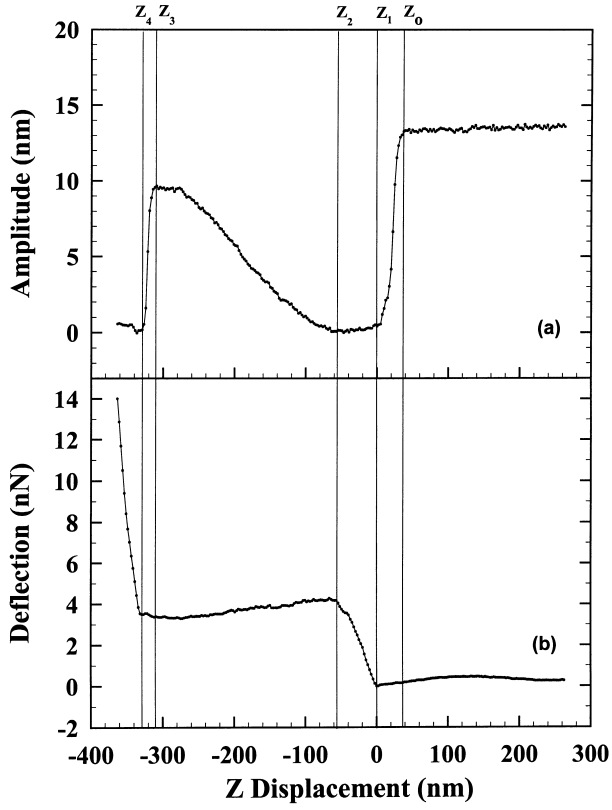

Figure 2. The change of amplitude (a) and deflection signal (b) recorded simultaneously during tip approach. The cantilever is driven by an oscillating magnetic force applied at the magnetic film coated at the back side. The tip is moved toward the sample surface with decreasing Z value. The values of the marked positions are $Z_0 = 34.4$ nm, $Z_1 = 0$, $Z_2 = -56.5$ nm, $Z_3 = -311.8$ nm and $Z_4 = -333.9$ nm, respectively. The deflection signal is converted to the force between the tip and the sample surface according to $F = kD$, where $k \sim 0.10$ Nm^{-1} is the spring constant of the cantilever and D is the deflection of the end of the cantilever in nanometers.

In the region where $Z > Z_0$, the cantilever freely oscillates with an amplitude of ~13 nm. When the tip of the nanotube is close to the mica surface, the oscillation amplitude starts to decrease. It drops to almost zero at $Z = Z_1$ (0 nm). As the tip approaches the surface, many complicated processes occur, including the magnetic force applied on the cantilever, the interaction between the tip and the surface, and the change of stiffness of cantilever, nanotube and sample surface.[11] Obviously, the decay of the oscillation amplitude with tip–sample distance in Fig. 2(a) is not linear. It can be divided into three parts according to the previous study on the force–distance curve of MAC Mode AFM in fluids.[11] Qualitatively, on initial contact, the oscillation amplitude decays exponentially to ~75% as Z decreases. At this stage, it is equivalent to non-contact mode measurements in which the tip senses the long-range attractive force from the surface. Presumably, the liquid between the tip and sample surface is stiffened by the interaction.[12] If the tip approaches further, the repulsion force starts to dominate and the amplitude decreases dramatically from ~75% to ~20%. The tip has firm contact with the mica surface

in this range. Thus the amplitude drops linearly with a slope $dA/dZ = 1$. The value of the amplitude in Fig. 2(a) is calibrated by using this linear part and assuming that $dA/dZ = 1$. We found that this treatment is satisfactory in explaining the results. At the third stage, the amplitude signal is almost zero but some residual amplitude is seen because the cantilever can still move even though both ends are now 'pinned'.

In the region between Z_1 and Z_2, the amplitude remains at zero but the deflection signal of the cantilever rises, indicating the increase of the force applied at the nanotube tip. At $Z = Z_2$ (−56.5 nm), the force applied onto the nanotube increases to ~4.2 nN. The deflection signal only slightly decreases but the amplitude clearly rises. This is due to the phenomenon of nanotube buckling at a certain contact force.[1,2] Once the buckling force is exceeded, the nanotube is bent easily with little additional force, thereby increasing the amplitude. The force applied to the nanotube tip is nearly constant in the range $Z_3 \leq Z \leq Z_2$. If the cantilever is moved closer towards the surface at $Z \leq Z_3$, the single nanotube extended out of the bundle is completely bent away so that the bundle touches the surface. The bundle acts as a rigid tip because it has a much higher buckling force. As a result, the oscillation amplitude quickly decreases to zero at $Z \geq Z_4$ and the deflection signal rises. The cantilever ($k = 0.10$ N m^{-1}) is much softer compared to carbon nanotube bundles, therefore buckling of the nanotube bundles was not observed within the experimental limits. If the cantilever is withdrawn after the nanotube bundle touches the surface, the single carbon nanotube was found to recover quickly to full length and identical force calibration curves can be obtained in successive cycles. The force–distance curves can be repeated again and again over several hours, demonstrating the resilience and reversibility of the nanotube AFM tips under a reasonable force load.

The unique reversible buckling of the carbon nanotube has been a very interesting research topic.[13,14] Applying single carbon nanotubes as AFM tips can be quantified easily with force–distance curves (buckling force), as shown in Fig. 2. The length of the single nanotube extended out of the bundle can be read directly from Fig. 2 as $L = Z_2 - Z_4 = 277$ nm. The diameter of the MWNT was characterized by other methods and is typically 5 nm (i.e. $r \sim 2.5$ nm). Assuming that the Young's modulus of the nanotube is similar to that of in-plane graphite ($Y \sim 1$ Tpa), the Euler buckling force of the nanotube with length $L = 277$ nm and radius $r \sim 2.5$ nm can be calculated as[1,2]

$$F_{\text{EULER}} = \pi^3 Y r^4 / 4L^2 \sim 4 \text{ nN}$$

This agrees very well with the force where the buckling starts in Fig. 2, therefore the force–distance curve and buckling force can be used to confirm directly the geometry of single-nanotube AFM tips.

Topography AFM images were taken by setting the feedback system to control the oscillation amplitude at a value of ~90% of the free oscillation value, namely at Z slightly below Z_0. As we discussed previously, the system actually worked in non-contact mode, therefore the interaction between the tip and the sample was minimized. The condition was maximized for measuring weakly adsorbed soft biomolecules at high resolution.

Figure 3 shows an image of lambda DNA molecules deposited on a piece of freshly cleaved mica from a 2 μg ml^{-1} solution. To enhance the bonding of DNA molecules to the mica surface, ~1 mM $MgCl_2$ was introduced into the solution. The image was taken in a 2.3×2.3 μm^2 area. Single DNA molecules were clearly resolved. Some DNA aggregates together, but most of the molecules were spread out on the mica surface. The height of these DNA stretches is ~2 nm, which corresponds to the real size of DNA molecules. The lateral resolution is ~5–8 nm, which is slightly worse than the 3 nm resolution of the best images measured with normal MAC Mode tips by taking advantage of small asperities

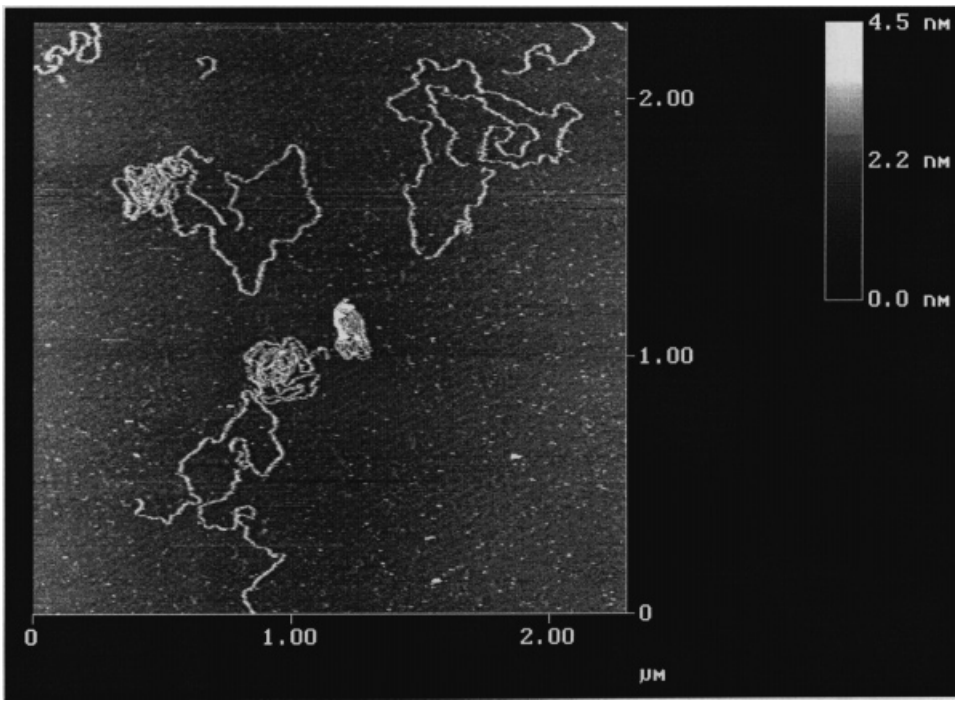

Figure 3. The AFM image of DNA molecules in 1 mM $MgCl_2$ buffer solution with a single carbon nanotube AFM tip.

on the tip.[8] However, the carbon nanotube tips did not show the multi-tip problem that is frequently encountered in using normal MAC Mode tips. Clearly, this is because the images were taken with a well-defined single carbon nanotube tip. The lateral resolution is defined by the diameter of MWNTs, which is ~5 nm in this study. It can be improved further by using nanotubes with smaller diameters. Ideally, a close-domed single-wall nanotube (SWNT) tip would provide a much higher resolution. It is possible to resolve the double-helix structure of DNA molecules if the resolution can be pushed to 1.2 nm by using the SWNT AFM tips.

In summary, we have successfully demonstrated that carbon nanotubes attached to MAC Mode AFM tips can be used in imaging soft biological samples in water solutions at molecular resolution. The length and buckling force of the single nanotube can be derived directly from the force calibration curve. Consistent high-resolution plasmid DNA images were obtained with carbon nanotube tips. The resolution was defined by the diameter of the nanotube. In the future, SWNT AFM tips could provide the highest possible resolution such that the double-helix structure of DNA can be resolved. This technique should have a critical impact on molecular biological studies.

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