Measuring, Shortening and Functionalizing Carbon Nanotube Tipped AFM Probes for DNA Sequencing

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Abstract—A method is presented to indirectly measure and shorten carbon nanotubes grown from the tips of atomic force microscopy (AFM) probes. The measurement component exploits the nanotubes ability to elastically buckle and requires only those signals and actuators available on a standard AFM. The shortening operation is facilitated by electric arcing using a conducting niobium substrate. The shortening operation produces stable carbon nanotubes at the tips of AFM probes which can then be functionalized for use as high-resolution, chemically-specific force probes. An application to AFM-based DNA sequencing is also discussed.

Index Terms—carbon nanotube, atomic force microscopy, nanotechnology, DNA

I. INTRODUCTION

Shortly after its invention by Binnig and Quate [1], the atomic force microscope (AFM) became a vital tool in the study of micro and nanoscopic features. Subsequently, the AFM has demonstrated its usefulness through many important scientific contributions. Its unique ability to record extremely high resolution images without regard to sample conductivity and its ability to be operated at standard laboratory temperatures and pressures distinguish it from other imaging techniques such as electron microscopy. Recently, the AFM has been used in molecular biology as a tool to study interactions between single molecules [2], [14], and it continues to facilitate discoveries in the semiconductor, MEMS and life science fields [19].

As progress in nanotechnology continues, demand for high resolution images has increased to the point where the limitations of atomic force microscope (AFM) imaging become impediments to further discovery. In order to increase the lateral resolution of AFM images, single-walled carbon nanotubes (CNT) have been grown or attached to AFM probes due to their very small diameters and robust mechanical properties [9]. However, current techniques for the growth of carbon nanotubes from AFM probes do not control for the length of the nanotube, and tubes that are too long can buckle under loads experienced during imaging, degrading image quality. Therefore, a need exists for a processing step in the manufacture of AFM probes that shortens the nanotube and measures its length.

The rest of this paper will describe our approach for the measurement and shortening operation of a single-walled carbon nanotube grown from a silicon AFM probe. An example of high-resolution AFM imaging is provided in the context of an AFM-based DNA sequencing method.

II. CARBON NANOTUBE PROBE PROCESSING: SHORTENING AND LENGTH ESTIMATION

Much of the excitement in carbon nanotube (CNT) research has been directed at the possible application of the novel material. Their small size and possibility of functionalization, or attachment of specific molecules, have led to the suggestion that CNTs can be used as chemical and biomolecular sensors [10], [22]. The measurement of nanoscopic surface features within high aspect ratio microfluidic channels have been performed by carbon nanotube-equipped AFM probes, and they have proven useful as a metrology tool for characterizing microfluidic devices [23].

Other applications focused in electronics and integrated circuits use CNTs as electrically conducting nanowires or as diodes [7], [25], and much of this effort is directed at controlled growth of nanowires [16], [21]. Also, high density storage devices (1.6 Tbits/in) have been demonstrated using a CNT that locally oxidizes an atomically flat sheet of titanium to write bits of data [8]. We turn our attention to the application of carbon nanotubes as high resolution, chemically-specific force probes for use in atomic force microscopy.

Single-walled carbon nanotube AFM probes are typically synthesized with a chemical vapor deposition (CVD) process that does not precisely control for the length of the nanotubes. A wafer of etched silicon AFM probes is placed in a CVD oven for processing, and nanotubes grow from catalyst particles in random directions. Once a growing nanotube reaches the apex of the AFM probe the nanotube will grow away from the tip (Figure 1, left). The length of the nanotubes is not regulated as part of the manufacturing process, and the nanotubes can be as long as 2 µm. In order to obtain good image quality, the nanotube must be shortened to minimize thermal-induced fluctuations at the free end. Therefore, shortening is required to achieve a nanotube stable to the forces experienced during imaging (Figure 1, right). Our approach for shortening the nanotubes is outlined briefly in the next section. In the section that follows, we discuss a novel technique to estimate the length of the nanotube using only the AFM and nanotube-
equipped probe by exploiting the elastic buckling property of carbon nanotubes.

A. Shortening CNT Probes

Carbon nanotube AFM probes are obtained from Veeco Instruments [26] grown with chemical vapor deposition on intermittent-contact probes (i.e., tapping mode probes). Gas molecules with kinetic energy proportional to temperature, strike the nanotube causing the end to vibrate; and these disturbances cause a mean-square displacement of the tip. The mean-square free end displacement of the tip $\langle \Delta x^2 \rangle$ is given as

$$\frac{1}{2} \kappa \langle \Delta x^2 \rangle = \frac{1}{2} k_B T$$

where $\kappa$ is the bending stiffness of the nanotube, $k_B$ is the Boltzmann constant, and $T$ is the temperature. For long as-grown nanotubes, the mean-square displacement of the nanotube tip is tens of nanometers—too high for a useful probe. Therefore, the long nanotubes grown from the AFM probes must be shortened to an appropriate length (see Figure 1). Fortunately, since functionalization of the carbon nanotube requires removing the end caps, attachment of carboxyl groups and removal of excess nanotube can be performed in the same step. The most popular technique of shortening the nanotube is removal of material assisted with high voltages [6], [31].

Carbon nanotube AFM probes are shortened in air by attaching electrodes to the cantilever and the substrate as shown in Figure 2. A sputtered niobium substrate (Nioprobe, Electron Microscopy Services [13]) is connected electrically to ground. The probe is connected to a positive DC signal between 5 and 20 V. The AFM cantilever is then oscillated near its resonant frequency and brought into momentary contact with the substrate causes arcing and removes material from the nanotube. Bringing the probe in momentary contact with the substrate causes arcing and removes material from the nanotube. Due to the electric potential applied, an arc is created at the gap, removing carbon near the end of the nanotube. Shortening the nanotube in air allows oxidization to occur at the free end, which forms carboxyl groups. Because the amount of carbon removed cannot be controlled, the length of the newly-shortened nanotube must be measured after each shortening operation, and if it is still too long, the process is repeated. The process used to measure the length of the nanotube is described in the next section.

B. Measuring Nanotube Length Using the AFM

A carbon nanotube that is too long will vibrate under thermal fluctuations, negatively impacting its capability to produce high-resolution images. For this reason, it is important to know how long the attached carbon nanotube is and that it is sufficiently short for imaging. Traditionally, the probe with attached nanotube is removed from the AFM and imaged directly with an auxiliary tool such as a scanning electron microscope. (The carbon nanotube AFM probes shown in Figure 1 were imaged in this manner.) However, we propose a novel measurement technique that uses only existing AFM signals and does not require the probe to be removed from the AFM (see Figure 3).

This new approach takes advantage of the elastic buckling property of carbon nanotubes previously demonstrated [29], and the sensitive cantilever deflection capability of the AFM. Measuring the length of the nanotube with this new technique requires operating the AFM in force-distance mode where the force required to deflect the cantilever is plotted against the piezo scanner’s vertical extension. With the nanotube and the substrate electrically grounded, the piezo is further extended, causing the cantilever to deflect. Additional extension will cause the nanotube to buckle and the cantilever to relax, and this sudden cantilever release is recorded by the AFM. The length of the nanotube is approximated by comparing the piezo extension before and after nanotube buckling.

1 Experimentally, we have found that nanotubes less than 300 nm are stable for imaging.
recorded. The piezo continues to extend, causing the cantilever to deflect and an increased axial load on the nanotube, and eventually the nanotube buckles. The piezo extends further until the tip of the etched silicon probe makes contact with the substrate and this final extension value is recorded. The difference between the initial and final extension values of the nanotube provides an estimated length of the nanotube (any residual deflection of the cantilever must be taken into account).

Importantly, this method facilitates the iterative nature of the AFM-based shortening operation described earlier because it is not required to remove the nanotube/cantilever assembly from the AFM. See Figure 3 for a schematic of this operation.

Experimental data of this approach is provided in Figure 4. The AFM’s sensor reading (measuring cantilever deflection) is plotted as a function of piezo extension. Initially, the piezo is extending through free air until the nanotube makes contact with the substrate (marked as point (1) on the figure). The cantilever begins to deflect, causing the nanotube to experience increased axial loading until buckling occurs indicated as point (2) on the figure. Importantly, if this shortening operation is performed in air, carboxyl molecules are formed at the opened end of the nanotube, providing a convenient molecule onto which further chemical modification can be performed. One possible functionalization procedure is described in the next section.

C. Functionalization Chemistry

Once the CNT is shortened to an appropriate length, and carboxyl molecules have formed at the free end [6], further modification can be performed. Since carboxyl chemistry is well understood in bulk quantities, many functionalization procedures are available [10], [11]. Our interest lies in DNA sequencing, and therefore we focus on the attachment of thymine to the carboxyl molecule. Toward that end, the following procedure is adapted from [30]:

An esterification reaction is used to attach thymidine (a chemical derivative of thymine) to a carboxyl group via carbodiimide. Specifically, the shortened nanotube probes are placed in 0.1 M MES (2-[N-morpholino]ethanesulfonic acid) (Sigma Aldrich [20]) pH 6.0 buffer containing 50 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (VWR [28]) and 50 mM thymidine (VWR) for two hours. The probes are then washed in 0.1 M MES buffer pH 6.0, 0.1 M NaCl (VWR), and deionized water. During this process, the carbodiimide attacks the primary alcohol group on the thymidine’s sugar. The nucleotide attached to the nanotube is expected to be stable.

III. APPLICATION TO DNA SEQUENCING

As an example of a need for the high resolution imaging provided by functionalized carbon nanotube probes, we present recent work on AFM-based DNA sequencing.

Since the announcement of the complete human genome, scientists have begun sifting through the tremendous amount of genetic data [17], [27]. While much progress has been made toward the understanding of the genetic foundation of our species, it must be emphasized that any conclusions drawn from data obtained from the Human Genome Project comes from a very small sample of individuals. However, if a technology could be developed that facilitates inexpensive and rapid DNA sequencing such that personal genomic information becomes available, dramatic advancements would occur in medical diagnosis, drug development and prescription, and the study of genes associated with complex traits or multigenic diseases.

This section describes the design of a novel system that provides rapid DNA sequence information based on proven atomic force microscope (AFM) technology (Figure 5-A) [12]. The premise is complementary nucleotides (adenine and thymine or cytosine and guanine) bind together with forces large enough to be detected by an AFM. A force probe coated or functionalized with a single DNA nucleotide (e.g. thymine) is scanned across single-stranded DNA immobilized on mica, and the locations of the complementary nucleotide (e.g. adenine) are measured (Figure 5-B). In the future, multiple functionalized probes will be scanned across the same strand to sequence DNA in one pass. Initial work focuses on demonstration of the principle, and therefore only one probe is scanned at a given time.

Because the nucleotides are spaced so closely on the DNA backbone (0.34 nm for β-form), spatial resolution of the
AFM requires special attention. Toward that end, a single-walled carbon nanotube is grown from the AFM probe, shortened and functionalized with a nucleotide to act as a high-resolution, chemically-specific force probe. Recent reports have shown that carbon nanotubes can be synthesized with diameters of 0.42 nm, and nanotubes with diameters as small as 0.33 nm are predicted to be stable [5], [24]. These ultrasmall nanotubes are of the order of the base pair spacing of nucleotides on a DNA backbone (Figure 5-C) and could potentially provide sufficient spatial resolution as to determine individual nucleotides on a target strand. Carbon nanotube AFM probes offer unprecedented resolution and recently have been functionalized with various chemical groups.

A. Experimental Results

To demonstrate the method, single-stranded DNA is imaged with a functionalized carbon nanotube probe. The attractive force interactions between the force probe’s nucleotide and the nucleotide’s complement on single-stranded DNA (ssDNA) are measured as an increase in phase lag of the oscillating AFM cantilever [3]. The approach is to immobilize ssDNA on atomically-flat mica, and scan a region of the mica with the AFM in tapping mode. A detailed explanation of the procedures used to prepare the synthetic DNA on mica for imaging are provided in [2], [4].

The experiments are performed on synthetic DNA fragments of 60 nucleotides, giving an expected length of 20 nm. The nanotube probe is shortened and functionalized with thymine, and scanned over ssDNA of different sequences in two experiments. The primary experiment scanned synthetic DNA of the sequence
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5'\text{-}25\text{A}-10\text{A}\text{-}25\text{T}-3'.
\]
It is expected to measure an increase in phase lag between the thymine-functionalized probe and the adenine molecules on the test strand at the center of the DNA fragments. A control experiment is also performed with the same thymine-functionalized probe on a synthetic strand of the sequence 5’-60T-3’.

The resulting data from these two experiments are presented in Figures 6 (primary) and 7 (control). For the case of the primary experiment, a test fragment of DNA is identified in the topography region of Figure 6 based on its length and height (20 nm and 1 nm, respectively). The corresponding location in the phase response shows an increase in phase lag (shown as a darker region) at the center of the strand where thymine is expected to interact with the 10 adenine nucleotides.

More explicitly, the sequence of the synthetic DNA (listed from the 5’ end of the deoxyribose molecule to the 3’ end) is 25 thymine, 10 adenine and 25 thymine nucleotides.

B. Future Implementation—Integrated Microfluidics Device

It is important to note that current experiments are performed in air which limits nucleotide bonding. To enhance the bonding force and improve the signal-to-noise ratio, it is desirable to conduct the probe–ssDNA interactions in solution where pH can be controlled. In fact, recent studies indicate that nucleotide interaction is a strong function of pH [15], and if these experiments were performed in liquid, an optimal pH can be selected that maximizes attractive forces between...
complementary pairs, and also maximizes repulsive forces between non-complementary pairs.

To further streamline sequencing, an integrated microfluidic device is envisioned that pumps single-stranded DNA in solution past four oscillating cantilevers, each functionalized with a different nucleotide. This MEMS device must include necessary channels and sensors necessary to route and orient DNA so that it is available for interrogation.

IV. CONCLUSION
Carbon nanotubes grown from the tips of AFM probes are measured via buckling and shortened with electrical arcing. Further chemical modification is performed to provide a high-resolution, chemically-specific force probe used in DNA sequencing. Early work is presented toward the demonstration of a novel sequencing technique by scanning single-stranded DNA with the functionalized carbon nanotube probe.

Future work will be directed at three major aspects of the described method: improving nanotube attachment procedures, exploring molecular models of the buckling process, and refining the functionalization protocols.

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Fig. 7. AFM data of the control experiment with the same thymine-functionalized probe. All fragments scanned in this figure have the sequence 5′-40yrv-3′. Top: A strand of ssDNA is identified by its length and height. Bottom: The phase response of the scanned region. Detail: The identified strand of DNA shows no variation in phase response along its length, indicating no preferential attractive forces.


