Carbon Nanotube Tips: High-Resolution Probes for Imaging Biological Systems

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Herein, we report the first atomic force microscopy (AFM) imaging studies with carbon nanotube tips that address their potential to improve lateral resolution and to probe biological systems. Multiwall carbon nanotubes (MWNTs) and single-wall carbon nanotubes (SWNTs) have been attached to the ends of single-crystal silicon cantilever tip assemblies and used to image amyloid β (1–40) derived protofibrils and fibrils by tapping mode AFM. Image analysis shows that the average resolution obtained with the nanotube tips is significantly better than that obtained with our best silicon tips and provides new insight into the structure and assembly mechanism of amyloid fibrils. The potential for imaging with molecular resolution using these tips is discussed.

The feature resolution obtained by AFM is determined in large part by the size and shape of the probe tip used for imaging.1,2 Commercially available probes consist of microfabricated pyramids of Si or Si3N4 that have end radii of curvature as small as 10 nm but are often much larger.2,3 These tips place significant constraints on potential lateral resolution, and furthermore, the pyramidal shape restricts the ability of these tips to access narrow and deep features. Small cone angle carbon protrusions with radii of 10–30 nm deposited on the ends of conventional tips significantly improve depth but not lateral resolution.4 Recently, a potential breakthrough in probe technology was achieved by attaching MWNTs to the ends of Si tips.5 In this work, the cylindrical geometry of the MWNT tips was exploited to image a deep grating with excellent fidelity, although potential improvements in lateral resolution were not investigated.

A typical scanning electron microscopy (SEM) image of a MWNT tip attached to a conventional single-crystal silicon cantilever tip assembly is shown in Figure 1.5,6 This MWNT tip extends ca. 1.8 μm from the end of the pyramidal Si tip to which it is attached. The diameter of the major portion of the nanotube tip, 100 nm, is much larger than a single MWNT and corresponds to a bundle of tightly packed tubes.7 The MWNT bundles attached to the Si probes have diameters of 75 ± 27 nm; attached SWNT bundles8 typically are smaller with diameters of 45 ± 8 nm. The high aspect ratio nanotube tips have obvious advantages for probing deep crevices and steep features.9 Additionally, it is

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Figure 1. SEM image of a MWNT tip attached to a silicon cantilever tip assembly. The inset corresponds to a higher magnification view that highlights the nanotube. The orientation of the inset is rotated 180° relative to the main image. The white bar corresponds to 1 μm.

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(6) MWNTs and SWNTs were prepared by arc discharge and laser vaporization methods, respectively. MWNT samples were purified by oxidation (700 °C, air) until ~2% of the original mass remained. SWNT samples were purified by sonication and filtration through 0.8 μm pore membranes. Nanotubes were attached to the pyramids of gold-coated Si cantilevers (FESP, k = 1–5 N/m), Digital Instruments, Inc., Santa Barbara, CA, using an acrylic adhesive (adhesive carbon tape, Electron Microscopy Sciences, Fort Washington, PA) under the direct view of an optical microscope. The as-made nanotube tips are typically too long for high-resolution imaging and were shortened by applying a bias voltage between the tip and a sputtered Nb surface.
(10) The buckling force, Fb, can be estimated from Fb = πEbh2/2 where E is Young’s modulus, I is the moment of inertia πr4/3, and L is the length of the unsupported nanotube. For typical radii r, L and E = 1.2 T Pa, the range of bucking forces will be 5–30 nN.
(15) All images were acquired with a Nanoscope III instrument (Digital Instruments, Santa Barbara, CA) under ambient conditions in tapping mode. All samples were prepared by depositing a 3–5 μL aliquot of Aβ(40) solution on freshly cleaved mica surfaces and then rinsing twice with 50 μL of water.12 Imaging parameters were optimized for individual tips; typical ranges for the FESP nanotube tips were (i) free RMS oscillation amplitude, 30–40 nm, (ii) setpoint voltage, 0.45–0.8 V, and (iii) scan rate, 0.25–1 Hz.

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lower arm of the Y approximately one-half of the height of the major fibril body. (2) a defect in the periodic structure (middle arrow), and (3) a staggered fibril end (right arrow). A critical difference between the present data of the Y-branch and previous studies\(^\text{14}\) is the decrease in size after branching. The Y-branch and staggered end are believed to correspond to active growth sites to which protofibrils add during fibril assembly\(^\text{13}\) and are thus indicative of the potential of nanotube tips for unraveling the growth mechanism of A\(_\beta\) fibrils. In addition, we find that the MWNT and SWNT tips are more robust and less prone to contamination than Si tips. Adhesion forces observed with our nanotube and SWNT probe tips. While further improvements in resolution will be needed in order to approach true molecular resolution imaging in air and in liquids, we believe that nanotube tips offer a rational pathway to do so in the future.

Table 1. Summary of Data Comparing the Resolution Obtained with Nanotube and Silicon Tips on A\(_\beta\)40 Fibrils and Protofibrils

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tip</th>
<th>Width at half-maximum (nm)(^a)</th>
<th>Depth between subunits (nm)(^b)</th>
<th>Tip radius (nm)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>type-1 fibril</td>
<td>MWNT</td>
<td>18.6 ± 2.2</td>
<td>3.2 ± 0.4</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>SWNT</td>
<td>11.9 ± 0.7</td>
<td>2.7 ± 0.4</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Si-TESP</td>
<td>26.0 ± 0.9</td>
<td>2.7 ± 0.3</td>
<td>19.7</td>
</tr>
<tr>
<td></td>
<td>Si-FESP</td>
<td>21.5 ± 1.8</td>
<td>2.5 ± 0.3</td>
<td>12.9</td>
</tr>
<tr>
<td>protofibril</td>
<td>MWNT</td>
<td>11.4 ± 0.4</td>
<td>1.1 ± 0.1</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>Si-TESP</td>
<td>14.4 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>Si-FESP</td>
<td>14.8 ± 1.7</td>
<td>0.7 ± 0.2</td>
<td>16.9</td>
</tr>
</tbody>
</table>

\(^a\) The widths at half-maximum were determined from cross-sections taken perpendicular to the fibril and protofibril axes. \(^b\) Maximum-to-minimum height variations determined from cross-sections taken along the periodic fibril/protofibril axes. \(^c\) The effective tip radii were calculated\(^\text{10}\) for diameters of the fibril and protofibril of 7.8 and 3.1 nm, respectively. \(^\text{12}\) The fibril and protofibril heights measured with the nanotube and Si tips were consistent with these diameters.

Figure 2. Typical AFM image of a type I\(^\text{12}\) A\(_\beta\)40 fibril acquired with a MWNT tip. The arrows highlight specific features discussed in the text. The white bar corresponds to 250 nm.

The improvement in resolution is due primarily to a reduction in the effective tip radii when imaging with nanotube tips. We have calculated the effective nanotube and Si tip radii by using standard deconvolution methods\(^\text{18}\) and compare these results in Table 1.\(^\text{19}\) These data highlight the improvements achieved with the nanotube tips. In particular, the average MWNT tips exhibit radii of 9 nm compared with 13–20 nm for Si.\(^\text{17}\) Even more impressive are the initial results obtained with SWNTs that show an average effective radius of only 3 nm. We believe that the use of SWNT tips is especially promising, since the observed radii are still substantially larger than the 0.5–0.7 nm radii of individual SWNTs;\(^\text{3}\) i.e., sharper tips will be achievable if methods to expose individual tubes at the tip ends are developed. These studies demonstrate for the first time that significantly improved lateral resolution is attainable on biological samples using MWNT and SWNT probe tips. While further improvements in resolution will be needed in order to approach true molecular resolution imaging in air and in liquids, we believe that nanotube tips offer a rational pathway to do so in the future.

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\(^\text{17}\) Similar results were obtained from the analysis of images of 5 and 10 nm Au colloids (Ted Pella, Redding, CA) recorded using nanotube and Si tips. Au colloids have been used previously as an imaging standard: Vesenka, J.; Manne, S.; Giberson, R.; Marsh, T.; Henderson, E. Biophys. J. \textit{1993}, \textit{65}, 992.