Scanning Microscopy

Volume 4 | Number 3 Article 2

8-23-1990

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Recommended Citation

Allison, D. P.; Thompson, J. R.; Jacobson, K. Bruce; Warmack, R. J.; and Ferrell, T. L. (1990) "Scanning Tunneling Microscopy and Spectroscopy of Plasmid DNA," *Scanning Microscopy*: Vol. 4 : No. 3 , Article 2.
Available at: https://digitalcommons.usu.edu/microscopy/vol4/iss3/2

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Scanning Microscopy, Vol. 4, No. 3, 1990 (Pages 517-522) 0891-7035/90$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SCANNING TUNNELING MICROSCOPY AND SPECTROSCOPY OF PLASMID DNA

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(Received for publication June 7, 1990, and in revised form August 23, 1990)

Abstract

We present scanning tunneling microscope (STM) images of uncoated deoxyribonucleic acid (DNA) electrochemically mounted on highly-oriented pyrolytic graphite (HOPG) and imaged in air. Images of linear abnormalities inherent to HOPG surfaces that can be confused with DNA are also presented. Scanning tunneling spectroscopic (STS) images generated by superimposing a small, high frequency ac bias onto the dc tunnel bias and recording the ac current signal were taken simultaneously with the topographic images. These spectroscopic images reveal contrast due to local conductivity variations and can be used to differentiate DNA molecules from graphite artifacts.

Key Words: Scanning tunneling microscopy, deoxyribonucleic acid, graphite, scanning tunneling spectroscopy, differential conductivity

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Introduction

Unlike the material sciences where scanning tunneling microscope (STM) images can be obtained directly from conducting or semiconducting surfaces, the successful application of STM technology to biological problems requires that samples be placed onto conductive surfaces. This presents three major problems.

First, the vertical roughness of the mounting surface cannot be larger than the vertical dimension of the sample. For example, palladium/gold (40:60 Pd/Au) sputter-coated onto a cleaved mica surface has a vertical roughness of 1-2.5nm and is an adequate substrate for mounting a relatively large biological sample like tobacco mosaic virus (TMV) with a vertical dimension of 18nm (Mantovani, *et al.* 1990). However, DNA molecules with vertical dimension of 2nm are easily hidden in this substrate and cannot be resolved. Although the first reported image of unstained DNA was mounted on a carbon substrate (Binnig and Rohrer, 1984), other studies have addressed this problem by using atomically flat surfaces of either gold (Lindsay and Barris, 1988) or highly-oriented pyrolytic graphite (HOPG) (Travaglini *et al.*, 1987, Beebe *et al.*, 1989, Lee *et al.*, 1989, Keller *et al.*, 1989), with the exception of Cricenti *et al.* (1989) who reported images of uncoated DNA molecules mounted on a gold plated aluminum stub.

Second, the sample must be anchored to the mounting surface so as not to destroy the integrity of the sample, but with sufficient strength to avoid displacing the sample by the mechanical and electrical forces exerted by the tunneling tip as it scans the sample. This problem is passively dealt with on studies with HOPG, since it is commonly accepted that DNA molecules imaged are either trapped by step edges or other imperfections in the surface, or are anchored by precipitated solids as the sample dries on the surface. The electrochemically mediated attachment of DNA to a gold surface (Lindsay and Barris, 1988, Lindsay *et al.*, 1989) is the only reported study of actively binding DNA to a substrate, although other laboratories, ours included, are exploring chemical modifications of surfaces for the purpose of DNA attachment.

Finally, the mounting surface topography should not have inherent features and or defects that either obscure or could be interpreted as part of the object being examined. Unfortunately, in both monatomic gold and HOPG substrates, shifts in the surface crystalline plane of less than 1 nm will result in step edges that are imaged in the STM

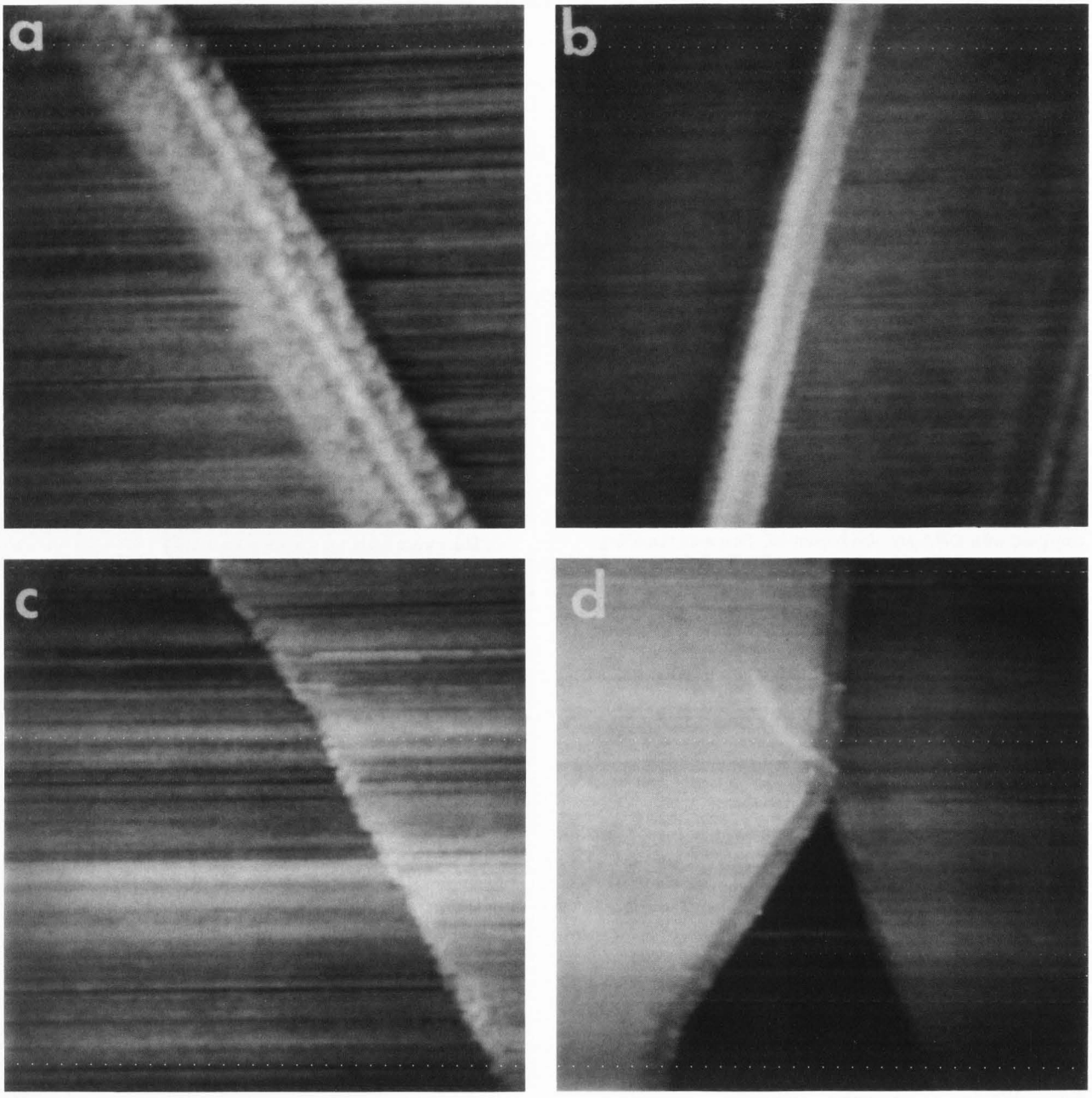

Figure 1. STM images of features on HOPG surfaces. Heights are shaded from black (lowest) to white (highest) in the ranges indicated. (a) DNA-treated sample. Height range is 2.9 nm. Fieldwidth = 50 nm. (b) Feature found on untreated HOPG. Height range is 6.4 nm. Fieldwidth = 100 nm. (c) Mono-atomic planar step on untreated HOPG. Height range shown is 2.2 nm. Fieldwidth = 100 nm. (d) Single and multiple steps found on HOPG treated with Tris buffer solution only. Height range shown is 2.1 nm. Field width = 200 nm.

as linear or irregular lines that may be confused with DNA molecules. In this report we describe the use of spectroscopic information (Binnig and Rohrer, 1982) taken simultaneously with topographic scans that can be used to help identify DNA molecules from linear abnormalities commonly found on HOPG surfaces.

Materials and Methods

Sample Preparation

A 10μl droplet of PZ189, a circular plasmid DNA, diluted to 40 μg/ml in 0.1M Tris + 0.001M EDTA at pH 7.5 was deposited onto a freshly cleaved HOPG surface.

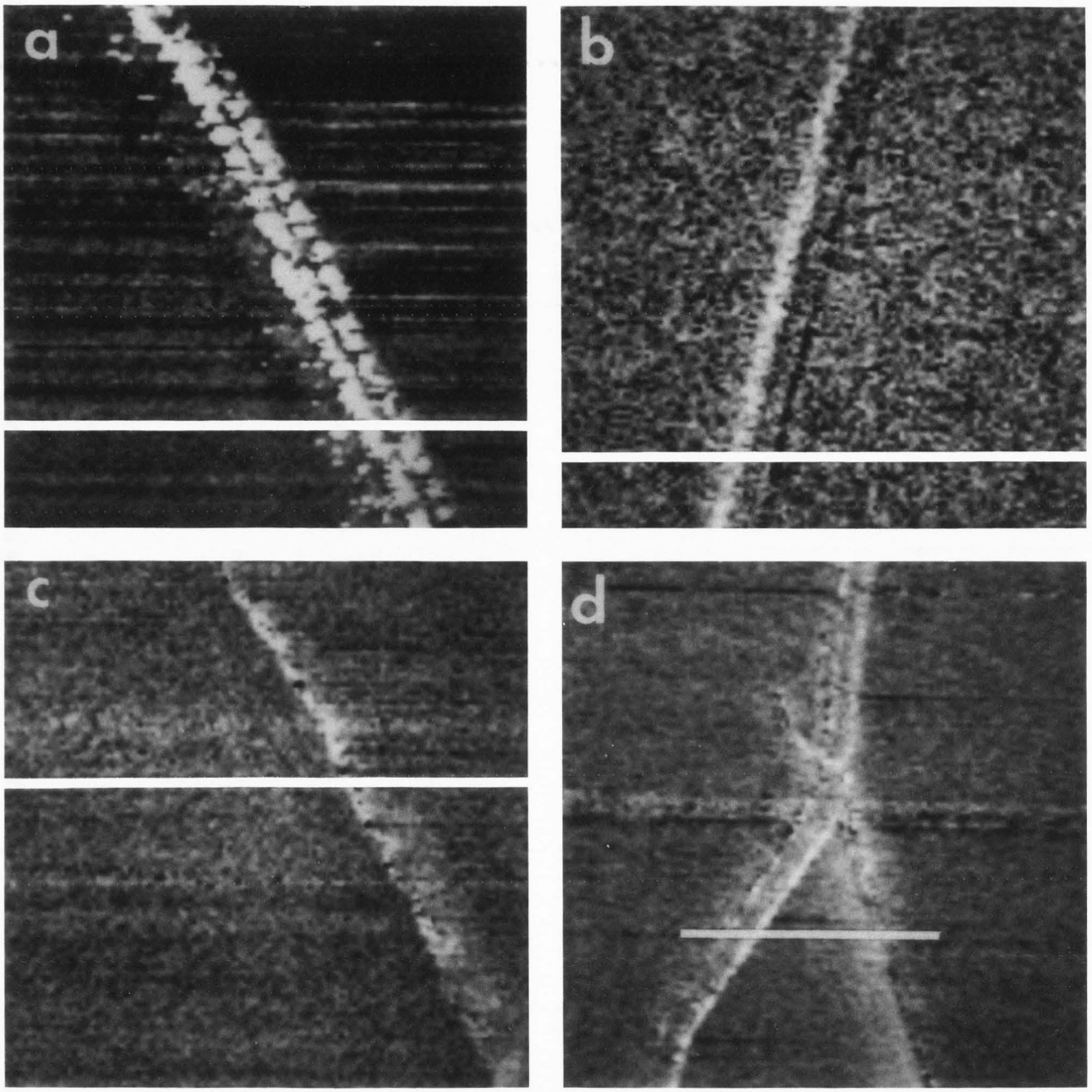

Figure 2. Spectroscopic images recorded simultaneously with the corresponding images of Fig. 1. White features are shown with *decreased* differential conductivity and are displayed on varying range scales to allow viewing of significant features. A direct comparison of the scales of features is shown in Fig. 3.

Electro-deposition was accomplished by contacting the droplet surface with a platinum electrode which was held at a potential of 2-4 V with respect to the graphite. This was maintained for three minutes. The unbound DNA was removed by touching the sample surface to a 200μl droplet of buffer solution followed by two similar rinses with distilled water. Excess liquid was removed by wicking with filter paper. As a control the identical procedure was done in an identical buffer solution without DNA.

Scanning Tunneling Microscopy

STM images were taken in air using a commercial STM, Nanoscope I (Digital Instruments, Santa Barbara, CA) interfaced with a computer to digitally control the raster pattern and acquire image data. The feedback circuit built into the instrument controlled the tip height (S) to keep a constant selected tunnel current (I). The raster was calibrated to better than 10% using images of the atomic structure of HOPG. The tip height signal from the piezo

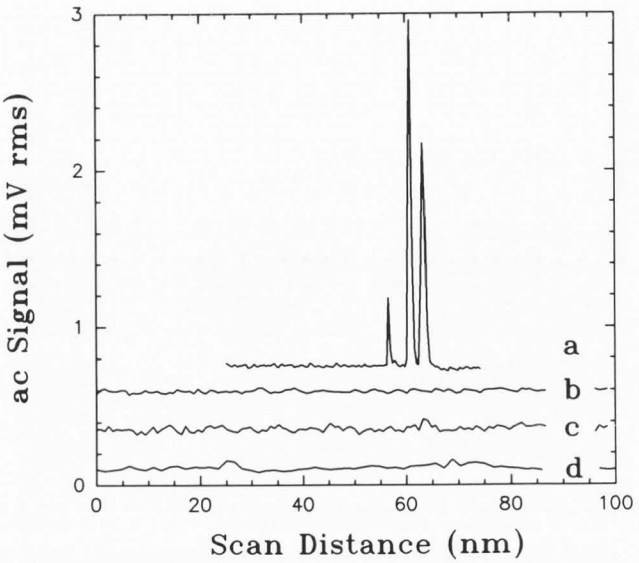

Figure 3. Spectroscopic signal amplitude as a function of position for typical horizontal scan lines in Fig. 2. Data shown in Fig. 3 was taken from the line displayed in Fig. 2. The ac bias signal in all cases was 7 mV rms. For clarity, the curves are shown with an arbitrary shift in vertical position.

voltage was conditioned, recorded, and displayed as a topographic image. Data were generally taken using 1 na of tunnel current with sample biases (V) of about 50 mV. The polarity of the bias did not appear to affect any of the results. Topographic images were recorded using frames of 128X128 points at 1-5 msec per point. Simultaneously, a spectroscopic signal was recorded in a parallel frame to display spatial images of conductance variations.

Generation of the spectroscopic images was accomplished as follows. A small high frequency ac bias (7 mV rms @ 100 kHz) was injected on the dc bias and the resulting ac tunnel-current signal was recorded with a single-channel lock-in amplifier. The high frequency was necessary so that the STM feedback circuit was not affected, thereby allowing normal constant-current control to be maintained. Even though the current amplifier was operating in the open-loop mode at low gain at this frequency, a sizeable (mostly capacitive) signal was observed. The spectroscopic images presented here were recorded at -90 degrees with respect to the phase of the signal maximum. The resulting output signal roughly corresponds to the negative of the local differential conductivity, i.e. $-dI/dV$. Thus high points are shaded white in the images and correspond to points of *low* differential conductivity. The features displayed in the images below reveal spectroscopic structure essentially independent of scan speed.

Results

A number of features were found on untreated HOPG substrates and on substrates treated with 0.1M Tris + 0.001 M EDTA buffer with and without DNA. Fig. 1 shows examples of features seen in topographic images. In Fig. 1a, a structure with a nodal period of about 2.3 nm is seen on a DNA-covered surface. The widths of each of the apparent strands is about 2.0 nm which is in agreement with the known width of DNA. However, the 2.3 nm nodal period is less than the 3.4 nm helical repeat expected with B-form DNA. Similar structures identified by having periodicities varying from 2.0 nm to 4.7 nm and strand widths from 2.0 to 4.0 nm were seen on numerous occasions, but at a greatly reduced frequency than calculated from the known surface densities of the applied DNA. Most often these stands were observed in close lateral associations and rarely in unassociated strands.

Occasional features such as narrow or broad ridges and trenches were also observed on untreated HOPG. An example is shown in Fig. 1b. Here an apparent double strand structure appeared on top of the basal plane of graphite. Each of the strands is about 4.0 nm in width and 2.0 nm in height. No periodic structure could be discerned. Other structures such as single and multiple atomic steps were frequently found on HOPG. In Fig. 1c a single atomic step is shown on untreated graphite while in Fig. 1d multiple steps and three distinct crystal planes can be seen on HOPG treated with buffer only.

Spectroscopic images ($-dI/dV$) corresponding to and taken simultaneously with the topographic images are presented in Fig. 2, with high points being shaded white and corresponding to points of low differential conductivity. A strong $-dI/dV$ signal over the DNA molecule in Fig. 2a indicates a profound difference in conductivity between the DNA and the HOPG substrate. Although a certain nodal periodicity can be detected in this image, the point to point resolution is not as good as the topographic image of Fig. 1a, and the 2.3 nm periodicity cannot be resolved. Spectroscopic images of anomalous structure (Fig. 2b) and step edges (Fig. 2c) on untreated HOPG and images of step edges obtained from graphite treated with buffer (Fig. 2d) are similar to one another but are much less intense than $-dI/dV$ images obtained over DNA molecules. In Fig. 3 the spectroscopic signal from a representative line in each of the images of Fig. 2 are plotted on the same scale for direct comparison. Fig. 3a clearly shows a marked difference in conductivity over the DNA molecules while imperfections in the graphite (Fig. 3b,c,d) can hardly be distinguished above the background.

Discussion

Most of the DNA molecules placed on HOPG surfaces are not detected by STM. This observation is consistent with reports of other researchers and in our own experiments using ^{32}P radio-labeled plasmid DNA electrochemically deposited. After rinsing and wicking off the excess liquid, more than enough DNA remains on the graphite surface to be easily seen by STM (Allison *et al.*, manuscript in preparation). Therefore one must conclude that DNA on HOPG is either invisible to STM examination, not present in the regions examined, or that the forces exerted by the tip sweeps the DNA off the graphite surface. However, there is ample evidence from our findings and those of others (Travaglini *et al.*, 1987, Beebe *et al.*, 1989, Lee *et al.*, 1989, Keller *et al.*, 1989), that at least some of the DNA structure is observable although most is found associated with imperfections in the graphite or trapped in step edges. Until more reliable techniques

are developed for routinely mounting and imaging DNA molecules on conductive surfaces, the researcher is presented with the problem of imaging occasional molecules that are often associated with step edges or other linear structure that can be confused with DNA.

The scanning tunneling microscope as first proposed by Binnig and Rohrer (1982) was developed as a means for imaging both topographic and electronic properties of conductors. Scanning tunneling spectroscopy has been used at the atomic level for identifying different density of states in both metal and semiconductor surfaces (Binnig *et al.*, 1983, Becker *et al.*, 1985a, de Lozanne *et al.*, 1985, Becker *et al.*, 1985b) and in combination with topographic images to gain a better understanding of structures. Travaglini *et al.* (1987), published the first STS image of DNA using *dI/dS* as a potential means for enhancing structural aspects of the molecule. Lindsay *et al.* (1988) used *dI/dS* measurements to identify adsorbate patches of DNA electrochemically mounted on atomically flat gold surfaces and imaged in a liquid environment.

In this study we have used *dI/dV* measurements at the molecular level as a means for qualitatively identifying DNA from the HOPG substrate and, more specifically, from imperfections in the substrate that could be confused with DNA. The lowered differential conductivity we have observed over the DNA is expected on the basis of its bulk conductivity and has also been reported by others (Lindsay *et al.*, 1988). Sharp reductions in differential conductivity have also been observed over larger biological specimens such as the tobacco mosaic virus (Warmack *et al.*, unpublished). More importantly, the spectroscopic imaging mode employed here provides a rapid way of discriminating qualitative differences which are not observable in constant current images alone. We have seen that spectroscopic contrast can be much greater than topological contrast and can be used as a primary search mode.

When and if reliable methods are developed for routinely mounting double and single stranded DNA to substrates, STS may evolve into an important tool for identifying individual bases by their unique electronic characteristics, thus providing a fast alternative method for sequencing DNA molecules.

Conclusion

Structures with topographic features consistent with their interpretation as DNA were observed by STM employing air-dried coatings on HOPG surfaces. Simultaneous images formed by using differential conductance showed remarkable contrast of these structures over the background substrate. Structural features on HOPG not exposed to DNA revealed only very weak spectroscopic signals. We conclude that spectroscopic imaging can be valuable in distinguishing artifacts which are occasionally observed on these substrates.

Acknowledgements

The authors are grateful to Drs. Gilbert M. Brown and Edward C. Uberbacher for their critical review of this manuscript and to Dr. Arthur Moore of Union Carbide Corporation for his generosity in supplying us with the HOPG substrates. We would also like to acknowledge the helpful discussions with Dr. Stuart M. Lindsay concerning the electro-deposition of DNA onto conducting surfaces. This research was sponsored by the Director's Research and Development Fund at ORNL and the Office of Health and Environmental Research, U.S. Department of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

References

Becker RS, Golovchenko JA, Hamann DR, Swartzentruber BS (1985a) Real-space Observation of Surface States on Si (111) 7X7 with the Tunneling Microscope. Phys Rev Lett **55**: 2032-2034.

Becker RS, Golovchenko JA, Swartzentruber BS (1985b) Electron Interferometry at Crystal Surfaces. Phys Rev Lett **55**: 987-990.

Beebe TP, Wilson TE, Ogletree DF, Katz JE, Balhorn R, Salmeron MB, Siekhaus WJ (1989) Direct Observation of Native DNA Structures with the Scanning Tunneling Microscope. Science **243**: 370-372.

Binnig G, Röhrer H (1982) Scanning Tunneling Microscopy. Helv Phys Acta **55**: 726-735.

Binnig G, Röhrer H, Gerber Ch, Weibel E (1983) 7X7 Reconstruction on Si (111) Resolved in real space. Phys Rev Lett **50**: 120-123.

Binnig G, Röhrer H (1984) Scanning Tunneling Microscopy. *Trends in Physics*, J Janta and J Pantoflicek (eds), European Physical Society The Hague, 38-46.

Cricenti A, Selci S, Felici AC, Generosi R, Gori E, Djaczenko W, Chiarotti G (1989) Molecular Structure of DNA by Scanning Tunneling Microscopy. Science **245**: 1226-1227.

de Lozanne AL, Elrod SA, Quate CF (1985) Spatial Variations in the superconductivity of Nb_3Sn Measured by low-temperature tunneling microscopy. Phys Rev Lett **54**: 2433-2436.

Keller D, Bustamante C, Keller RW (1989) Imaging of single uncoated DNA molecules by scanning tunneling microscopy. Proc Natl Acad Sci USA **86**: 5356-5360.

Lee G, Arscott PG, Bloomfield VA, Evans DF (1989) Scanning Tunneling Microscopy of Nucleic Acids. Science **244**: 475-477.

Lindsay SM, Barris B (1988) Imaging deoxyribose nucleic acid molecules on a metal surface under water by scanning tunneling microscopy. J Vac Sci Technol A **6**: 544-547.

Lindsay SM, Thundat T, Nagahara L (1988) Adsorbate deformation as a contrast mechanism in STM images of biopolymers in an aqueous environment: images of the unstained, hydrated DNA double helix. J Microsc **152**: 213-220.

Lindsay SM, Thundat T, Nagahara L, Knipping U, Rill RL (1989) Images of the DNA Double Helix in Water. Science **244**: 1063-1064.

Mantovani JG, Allison DP, Warmack RJ, Ferrell TL, Ford JR, Manos RE, Thompson JR, Reddick BB, and Jacobson KB (1990) Scanning tunnelling microscopy of tobacco mosaic virus on evaporated and sputter-coated palladium/gold substrates. J Microsc **158**: 109-116.

Travaglini G, Röhrer H, Amrein M, Gross H (1987) Scanning Tunneling Microscopy on Biological Matter. Surf Sci **181**: 380-390.

Discussion with Reviewers

G. Binnig and W. Heckl: It is known that steplines of graphite appear in the STM very differently. All kinds of quasi one-dimensional structures that falsely can be interpreted as DNA chains are observed. The observation of a strong dI/dV signal is no proof that DNA has been observed. First, topographic features generally modify also the dI/dV signal and dependend on the character of the multi-stepline this might be a more or less strong effect. Second, all kind of material could have agglomerated at the stepline. Please comment.

Authors: We agree that topographic images modify the dI/dV signal and that multi-steplines can produce more or less strong effects, in fact we have included three such images (Figure 2 b,c,d) in this paper. We also agree that all kinds of material can agglomerate at the stepline since it is well known that breaks or imperfections in the basal plane of graphite, which is otherwise chemically inert, exposes functional groups where chemical reactions can occur. Our dI/dV images on untreated graphite and graphite deliberately contaminated with Tris-EDTA buffer without DNA were controls intended to detect potential contamination of steplines with material other than DNA. Finally the dI/dV signal we observe over the DNA molecules, which are not associated with step edges, is more than an order of magnitude greater (Figure 3) than signals we observe over steplines and other imperfections in the graphite, including graphite treated with tris-EDTA buffer. Certainly a strong dI/dV signal over a molecule that appears to be DNA is persuasive evidence that the molecule is DNA likewise the absence of a strong dI/dV signal over a structure that looks like DNA would indicate that the molecule is an artefact.

G. Binnig and W. Heckl: We believe that it is worth while to publish a paper on STM-DNA only if it is definitely possible to distinguish different types of DNA molecules either topographically or with the aid of an additional technique, e.g. spectroscopy. Only this would then reveal valuable new information for biology. Please comment.

Authors: The problem of differentiating DNA molecules from artefacts inherent in graphite surfaces is a major problem recognized by our laboratory and other laboratories using the STM to image DNA. The reviewers also recognize the problem when they state, "It is known that steplines of graphite appear very differently. All kinds of quasi one-dimensional structures that falsely can be interpreted as DNA chains are observed." Therefore, any technique that can separate biological molecules from substrate artefacts should prove useful.

R. Balhorn: While the spectroscopy results appear to confirm the idea that the linear structures observed in Figs 1a and 2a are different from the carbon steps in 1b,c,d and 2b,c,d the reader is not presented with sufficient evidence that the structures analyzed in Fig 1a and 2a are really DNA. The diameter is similar but the periodicity is not close to that of B DNA (3.38). It would appear even a little short for A form DNA (2.57), the more likely conformation to exist under these conditions. Since the authors report using a circular DNA for the analysis, are there lower resolution images that can be used to show that they are indeed looking at part of a circle? Or at least demonstrate that the structure being imaged is curved and different from the linear structures too often found on graphite and mistaken for DNA. If there is any other evidence that can be provided to convince the reader that the strands being imaged are in fact DNA, it should be included. Otherwise the basic result being described, that spectroscopy can be used to discriminate DNA from graphite, is not really being proved. All that is being demonstrated is that this particular structure, whatever it is, appears differently from other steps. Please comment.

Authors: We do not have an image of the entire circular plasmid although this is one of our goals and we presume this is also a goal of other laboratories e.g. Cricenti et al. that are working with plasmids or other DNA molecules with molecular lengths of 2-3 μm and have not shown images of the entire molecule. We feel that important future work with DNA will require the development of reliable technique for the immobilization of DNA on substrates. The image we have presented of DNA has the right width to be DNA, the helix is right-handed, the helical repeat is consistent with A form DNA, the image is not on a graphite step and the dI/dV image is not consistent with images taken over imperfections in graphite but is consistent with what would be expected over a poorly conductive molecule such as DNA.