

2005, *109*, 14270—14274 Published on Web 07/07/2005

The Puzzle of Contrast Inversion in DNA STM Imaging

Errez Shapir,[†] Juyeon Yi,^{‡,§,*} Hezy Cohen,[†] Alexander B. Kotlyar,^{||} Gianaurelio Cuniberti,[§] and Danny Porath*,[†],⊥

Physical Chemistry Department, The Hebrew University, Jerusalem 91904, Israel, Physics Department, Pusan National University, Korea, Institute for Theoretical Physics, University of Regensburg, D-93040, Regensburg, Germany, Department of Biochemistry, Tel Aviv University, Ramat Aviv, Israel, and The Center for Nanoscience and Nanotechnology, The Hebrew University, Jerusalem 91904, Israel

Received: May 24, 2005; In Final Form: June 2, 2005

DNA has been at the center of an imaging effort since the invention of the scanning tunneling microscope (STM). In some of the STM imaging reports the molecules appeared with negative contrast, i.e., "submerged" under the metal background and darker. We demonstrate the phenomenon of contrast inversion in DNA STM imaging by controlled and spontaneous contrast inversions and by the dependence of the DNA apparent height with respect to the surface on the imaging bias voltage. Using these characterizations, we formulate a model explaining the above phenomenon by resonant tunneling through virtual states in the vacuum between the STM tip and the DNA molecule.

Negative contrast was observed¹⁻⁷ in few of the works reported in the past 15 years on DNA imaging using STM.⁸⁻²² Contrast inversion or change was never demonstrated in a controlled way, although in some reports the contrast was inverted unintentionally. According to the WKB theory, commonly used for STM images interpretation,²³⁻²⁵ the observed contrast inversion would imply that the DNA work function is lower than the surrounding metal, in dissonance with common understanding. Lindsay and others^{1,3-6} suggested possible mechanisms for the contrast inversion. Supporting control measurements were, however, difficult to perform at that time. Detailed calculation of a tip-DNA molecule-substrate system is even more difficult to calculate explicitly due to the complexity of the system. Therefore, the "contrast puzzle" in DNA STM imaging still calls for an elucidation.

We present constant-current STM measurements²⁶ of DNA molecules deposited on a flame-annealed gold surface²⁷ that show opposite imaging contrasts. Results as those reported below were observed for five different samples of poly(dG)-poly(dC), G4-DNA²⁸ and native dsDNA with no significant difference in behavior. The synthesized DNA was prepared by an enzymatic reaction that was characterized by absorption and CD spectra and by gel electrophoresis. Parts of the DNA molecules appeared higher than the surrounding gold (positive contrast) and others appeared lower (negative contrast). Contrast inversions from image to image, or during imaging, were achieved in a controlled way or spontaneously. The contrast was modified by changing the bias voltage as well. We present a critical choice of images to formulate a rationale of the

experimental conditions for observing the contrast inversion and to infer that contrast inversion is not attributable to structural deformations. We then propose a model explaining the physical origin of the puzzling results.

Figure 1 demonstrates contrast inversions; one inversion is spontaneous but the other three were obtained in a controlled way by changing the current set point. The images were scanned line by line (fast scan direction) downward or upward (slow scan direction) with a bias voltage setting, $V_b = 2.8 \text{ V}$. The image in Figure 1a is scanned downward (black arrows on the left side of the images), with the current set initially at $I_s = 0.5$ nA. In the upper quarter of the image, the DNA²⁹ appeared with positive contrast and then, without any modification of the imaging parameters, its contrast spontaneously changed to negative. After scanning another quarter of the image, the current was deliberately set down to 20 pA (the tip retracted) and the molecule contrast became positive again. The two insets show cross sections (marked by bars with corresponding colors) taken on the molecules at the negative (lower inset) and positive (upper inset) contrast parts. The image in Figure 1b (same area) was subsequently scanned upward, but now with $I_s = 0.5$ nA, and the contrast appeared negative. Near the same line where the current set was changed in the previous image, it was now changed back to 20 pA and the contrast became positive again. In these two images the positive contrast is observed at low current (tip far away) and the negative contrast at a higher current (tip close to the surface). This was always the case, except for spontaneous changes that occurred without changing the current or voltage settings. In Figure 1c the same area is scanned downward again with $I_s = 20$ pA. The molecule appeared intact and with positive contrast, indicating that the contrast inversion is an electronic rather than a mechanical effect. High voltage, in which the DNA appears at its maximum observed height but induce rough scanning conditions, was necessary because DNA molecules were invisible at lower (<1

^{*} Corresponding author. E-mail: Porath@chem.ch.huji.ac.il, Tel: +972-2-658-6948 and jyi@pusan.ac.kr.

[†] Physical Chemistry Department, The Hebrew University.

[‡] Physics Department, Pusan National University.

[§] Institute for Theoretical Physics, University of Regensburg.

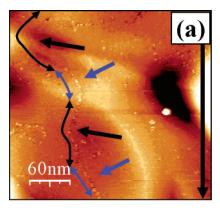
^{||} Tel Aviv University.

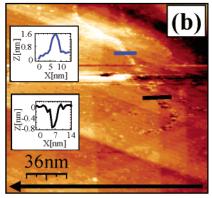
¹ Center for Nanoscience and Nanotechnology, The Hebrew University.

Figure 1. STM images (voltage setting, $V_b = 2.8 \text{ V}$, T = 300 K) showing contrast inversions, induced by changing the current setting. (a) The image was scanned downward (black arrows, left to the images, mark the slow scan directions). A spontaneous contrast inversion from positive to negative occurred, probably due to tip contact with the sample (white blob marked with a blue circle). Contrast inversion is induced by reducing the current set from $I_s = 0.5$ nA down to 20 pA (line is marked with a horizontal yellow line). The insets show the cross sections taken at the blue and black mark positions for the positive and negative parts, respectively. (b) Subsequent image, showing an opposite contrast inversion, scanned upward starting with $I_s = 0.5$ nA. Again, upon decreasing I_s from 0.5 nA to 20 pA the contrast is inverted from negative to positive. (c) Subsequent image, scanned downward with $I_s = 20$ pA, shows that the molecule was physically intact. The inset in Figure 1c shows a molecule in higher magnification and resolution, where the DNA pitch can be clearly seen.

V) voltages, possibly due to the large energy difference between the DNA LUMO and the Fermi level at the metal electrode. Deliberately induced contrast inversions were successful only in part of the attempts, clearly indicating that the current changes alone are not enough to induce contrast inversion.

Figure 2 shows spontaneous contrast inversions that, though rare, were observed several times. In the case, shown in Figure 2a, we observed three contrast inversions in one frame. The black and blue arrows indicate the negative and positive contrast parts, respectively. Figure 2a shows spontaneous contrast inversions similar to the inversion in the upper quarter of Figure 1a, but obtained several times within the same image, and with different voltage and current values than those of Figure 1a. It





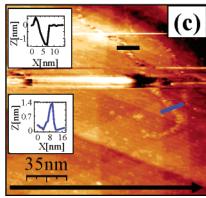


Figure 2. STM images showing spontaneous contrast inversions $(V_b = 2.5 \text{ V}, T = 300 \text{ K})$. (a) Three spontaneous contrast inversions observed in one image (scanned downward, vertical black arrow) marked by the arrows: negative (black) and positive (blue) contrasts $(I_s = 50 \text{ pA})$. Figures (b) and (c) are the forward (left) and backward (right) scans (scan direction is marked by the horizontal black arrow) in which opposite (spontaneous) contrasts are observed. Note also the mixed contrast in some parts of the DNA. The insets are cross sections marked by the nearest bars with the corresponding colors.

demonstrates that the contrast can change even without modifying the current set point in a variety of STM conditions: In particular, we observed them not only for the high current $I_s = 0.5$ nA (Figure 1a), but also for a moderately low current $I_s = 50$ pA (Figure 2a). Figures 2b and 2c show spontaneous contrast inversions of a different kind. Figure 2b was the left scan and 2c the right scan of one image (scanned downward with voltage $V_b = 2.5$ V and current set $I_s = 30$ pA). The lines in each image were acquired in an alternating way (black arrows indicate the fast scan direction). Here, an opposite contrast was observed in each scan direction, alternating every line. The contrast in these images appeared mixed in parts of the imaged molecule. Moreover, the sign of the contrast alters around the middle of the image, at a point where contact with the molecule might

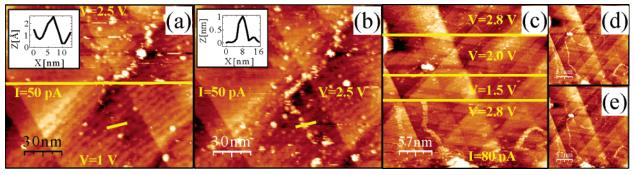


Figure 3. STM images showing the dependence of the apparent height on the bias voltage, V_b , without changing the current set, I_s , during the imaging. (a) The upper half of the image was scanned with $V_b = 2.5$ V. The bias voltage was turned down to 1 V, followed by a reduction of the apparent height of the molecules from 8 Å to 2 Å ($I_s = 50$ pA). (b) Subsequent image, scanned again with $V_b = 2.5$ V, where the apparent height is recovered. The cross sections in the insets of (a) and (b) indicate the apparent height at the different bias voltages. (c) In this image, the bias voltage was gradually changed downward and upward in a few positions along the slow scan direction, with the measured apparent height reacting to the bias voltage change. ($I_s = 80$ pA) Figures (d) and (e) were measured prior to and after the image in (c), showing that the apparent height change is electronic in origin. ((a) and (b) were measured at 300 K and (c)—(e) at 78 K).

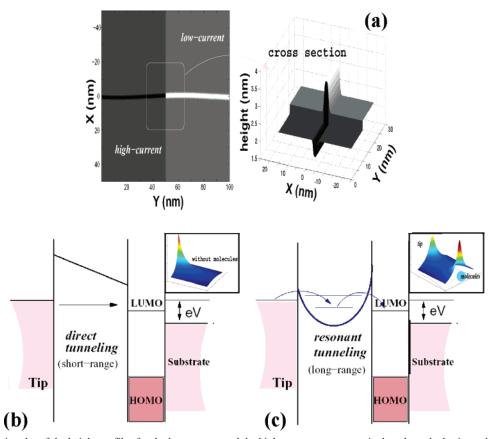


Figure 4. (a) A density plot of the height profiles for the low-current and the high-current sets respectively, where the horizontal thin line, exhibiting the change from black to white, represents the calculated tip movement at the positions of a molecule. The inset corresponds to three-dimensional blow-up of the region square-marked. The flat profile, corresponding to height measured on the gold substrate, displays tiny changes in height, and the downward and the upward bulges are reflect the contrast inversion in the molecule-lying region. (b) A schematic drawing showing the flat vacuum in the WKB picture and (c) the effective collapse of the vacuum barrier due to the presence of localized virtual states formed between the tip and the molecule.

have occurred. The insets show cross sections taken on the molecule, denoted by the blue and black marks close to them, which emphasize the alternating depression/protrusion. This inversion can be related to a distorted or asymmetric shape of the tip end that may have affected the field lines between the tip and the molecule. Note that the contrast inversion does not originate from some contamination adsorbed on the tip, because then the contrast is unlikely to change in opposite scan directions. A shift of the last atom in the tip apex could, in principle, change the current and induce a tip movement resulting in contrast inversion. In our experiments, however,

contrast inversion was observed solely on the DNA, making the above origin for the contrast inversion in our measurements unlikely.

Figure 3 demonstrates the effect of the bias voltage on the apparent height of the molecule. Figures 3a and 3b show a molecule that was measured with a current set $I_s = 50$ pA. The voltage was changed from 2.5 V in the upper part of the image to 1 V in the lower part, followed by an average decrease of the apparent height of the molecule from \sim 8 Å to \sim 2 Å. Figure 3b was measured right after the image in 3a, and the apparent height of the molecule is about the same all over the image,

and similar to the upper part of 3a. A similar image was acquired just before the image in 3a. This series indicates again that the height-voltage dependence as well as the contrast inversion at different current values is electronic in nature and not due to any morphological change in the molecule. Figure 3c shows the same effect in a more controlled way. Here, the voltage was changed from 2.8 to 2.0 V, followed by a considerable decrease of the apparent height, then to 1.5 V where the molecule is nearly unobservable, and back to 2.8 V with a recovery of the apparent height. Figures 3d and 3e were measured before and after the image in 3c, respectively, showing again with their similarity that the molecule was physically intact. Again, no contamination could interfere in this controlled series. Note that the tip height above the gold at the reported scanning conditions is estimated to be $\sim 1.5-1.8$ nm so upon approaching the molecule it does not crash into it in many of the cases, although some crashes were observed as well.

A theoretical simulation of STM measurements can be performed on the basis of the current formula: 30 I = $e/h \int dE[f(E) - f(E + eV)]T(E)$. Here f(E) is the Fermi-Dirac distribution function of an electron with energy E, and V is the voltage bias between tip and substrate. For currents from the tip to the gold substrate, invoking the WKB picture for the tunneling amplitude, $T \approx \exp[-2\sqrt{2mW/\hbar^2 z}] \equiv \exp[-z/\lambda]$, we obtain the conventional exponentially decaying tunneling current, as the tip-substrate distance z increases. With a typical value of the work function, W = 4 eV, we find the decay length for the gold, $\lambda \approx 3$ Å. The observed high corrugation (due to the contrast inversion of the DNA) implies a larger decay length. Within the WKB picture where the decay length is determined by the inverse of the work function, this would indicate that the work function of the DNA is smaller than that of gold, which is, however, unlikely (see Figure 1). Conceiving T explicitly for the tip-to-molecule tunneling is, in fact, highly complicated. The energy-level structure of molecules would definitely come into play in determining the current traits. In addition, possible nonequilibrium processes in the presence of the high voltage bias can be another factor of influence. We postulate here that as far as the long-range nature of the current is concerned, such particulars are not the major root of the contrast inversion. Instead, we assume localized states in the near-space between the tip and the molecule. Considering the husk of water, salt, and counterions added during the sample preparation and left encompassing the molecule, one can envisage a potential landscape that can possibly host states, shown pictorially in Figure 4d. In the factor T, we now take into account possible resonant tunneling through states accommodated in a potential well between the tip and the molecule. For the discussion here, we were mainly interested in a qualitative explanation. Without specifying the fine structure of such states, remaining in the simplest model, we regard a single energy level ϵ_v and resonant tunneling factor: $T(E) = \Gamma^2/(E - \epsilon_v)^2 + \Gamma^2$. According to the aforementioned current formula, we compute the height as a function of the lateral position, presented in Figure 4b. The results are indeed in accordance with our experimental observations. At high current, the tip gets closer to the molecule to keep the current constant (left side of the 2D plot, front side of the 3D inset). At low current, the tip retracts from the molecule (right side of the 2D plot, backside of the 3D inset).

The immediate cross-link between the above model and the experimental results is made through the formation of field lines between the tip and the molecule that can accommodate virtual states, enabling the long-range natured tunneling. This accounts for the contrast inversion regulated by the current set-point.

Furthermore, those field lines are possibly affected by changes in the tip apex shape that can even demolish the virtual states, which, a fortiori, would be responsible for the spontaneous contrast inversions shown in Figure 2. When objects with a regular shape and charge distribution are imaged, the tip-tosample potential takes the shape of flat lines, ruling out any localized states, such that contrast inversion is not observed. A shift of the last atom in the tip apex could, in principle, change the current and induce a tip movement resulting in contrast inversion. Such an inversion was, however, observed solely on the DNA in our experiments, making this origin for the contrast inversion in our measurements unlikely.

This work demonstrates the phenomenon of contrast inversion in STM imaging of DNA, both due to a controlled change in the imaging parameters or spontaneously induced. In addition, we show the dependence of the apparent height of the molecule on the voltage settings. Based on the experimental evidence, we formulated a model which describes the phenomenon via indirect tunneling through virtual states in the vacuum between the STM tip and the DNA molecule that are created by the curvature of the field lines due to irregular charge distribution on the tip and the molecule. This model accounts well for the observed phenomenon.

Acknowledgment. E.S. and J.Y. contributed equally to this work. We thank Igor Brodsky, Rosa Di Felice, Giovanni Constantini and Bernhard Knühl for assistance and fruitful discussions. The work was supported by the FIRST foundation of the Israel Academy of Science and Humanities, the German Israel Foundation, the European grant for Future & Emerging Technologies (IST-2001-38951), The Volkswagen Foundation, and Korea Research Foundation (KRF-2004-005-C00044).

References and Notes

- (1) Allison, D. P.; Bottomley, L. A.; Thundat, T.; Brown, G. M.; Woychik, R. P.; Schrick, J. J.; Bruce Jacobson, K.; Warmack R. J. Proc. Natl. Acad. Sci. U.S.A. 1992, 89, 10129-10133.
- (2) Nieminen, J.; Lahti, S.; Paavilainen, S.; Morgenstern, A. Phys. Rev. B 2002, 66, 165421-165429.
- (3) Lindsay, S. M.; Barris, B. J. Vac. Sci. Technol. A 1988, 6, 544-
- (4) Thundat, T.; Nagahara, L. A.; Oden, P.; Lindsay, S. M. J. Vac. Sci. Technol. A 1990, 8, 645-647.
- (5) Bottomley, L. A.; Haseltine, J. N. J. Vac. Sci. Technol. A 1992, 10, 591-595.
- (6) Allison, D. P.; Thundat, T.; Jacobson, K. B.; Bottomley, L. A.; Warmack, R. J. J. Vac. Sci. Technol. A 1993, 11, 816-819.
- (7) Kawai, T.; Tanaka, H.; Nakagawa, T. Surf. Sci. 1997, 386, 124-
- (8) Youngquist, M. G.; Driscoll, R. J.; Coley, T. R.; Goddard, W. A.; Baldeschwieler, J. D. J. Vac. Sci. Technol. B 1991, 9, 1304-1308.
- (9) Cricenti, A.; Selci, S.; Chiarotti, G.; Amaldi, F. J. Vac. Sci. Technol. B 1991, 9, 1285-1287.
- (10) Lindsay, S. M.; Lyubchenko, Y. L.; Tao, N. J.; Li, Y. Q.; Oden, P. I.; DeRose, J. A.; Pan, J. J. Vac. Sci. Technol. A 1993, 11, 808-815.
- (11) Zareie, M. H.; Philip, B. L. Biochem. Biophys. Res. Commun. 2003, 303, 153-159.
- (12) Selci, S.; Cricenti, A.; Felici, A. C.; Generosi, R.; Gori, E.; Djaczenko, W.; Chiarotti, G. J. Vac. Sci. Technol. A 1990, 8, 642-644.
- (13) Keller, W. R.; Dunlap, D. D.; Bustamente, C.; Keller, D. J.; Garcia, R. G.; Gray, C.; Maestre, M. F. J. Vac. Sci. Technol. A 1990, 8, 706-712.
- (14) Wilson, T. E.; Murray, M. N.; Ogletree, D. F.; Bednarski, M. D.; Cantor, C. R.; Salmeron, M. B. J. Vac. Sci. Technol. B 1991, 9, 1171-
- (15) Jing, W. T.; Jeffrey, A. M.; DeRose, J. A.; Lyubchenko, Y. L.; Shlyakhtenko, L. S.; Harrington, R. E.; Appella, E.; Larsen, J.; Vaught, A.; Rekesh, D.; Lu, F.-X.; Lindsay, S. M. Proc. Natl. Acad. Sci. U.S.A. **1993**, 90, 8934-8938.
- (16) Kanno, T.; Tanaka, H.; Nakamura, T.; Tabata, H.; Kawai, T. Jpn. J. Appl. Phys. 1999, 38, L606-L607.

- (17) Wang, H.; Tang, Z.; Li, Z.; Wang, E. Surf. Sci. Lett. **2001**, 480, L389-L394.
- (18) Tanaka, S. I.; Fujiwara, S.; Tanaka, H.; Taniguchi, M.; Tabata, H.; Fukuic, K.; Kawai, T. *Chem. Commun.* **2002**, *20*, 2330–2331.
- (19) Nishimura, M.; Tanaka, H.; Kawai, T. *Jpn. J. Appl. Phys.* **2002**, *41*, 7510–7511.
- (20) Terada, Y.; Choi, B. K.; Heike, S.; Fujimori, M.; Hashizume, T. *Nano Lett.* **2003**, *3*, 527–531.
- (21) Donato, M. C.; Jacqueline, K. B. J. Am. Chem. Soc. 2003, 125, 14964–14965.
 - (22) Tanaka, H.; Kawai, T. Surf. Sci. Lett. 2003, 539, L531-L536.
- (23) Wiesendanger, R., Scanning probe microscopy and spectroscopy; Cambridge University Press: Cambridge, 1994.
- (24) Meyer, E.; Hug, H. J.; Bennewitz, R. Scanning Probe Microscopy; Springer: Berlin, 2004.
- (25) Wolf, E. L. *Principles of Electron Tunneling Spectroscopy*; Oxford Science: London, 1989.
- (26) The STM measurements were performed at various temperatures (\sim 300 K, 77 and 4 K) in ultrahigh vacuum (UHV, \sim 5 × 10⁻¹¹ mbar), using a commercial STM (Omicron LTSTM). In all the measurements,

- topography and current were acquired simultaneously. Data analysis was performed with the freeware WSxM software (www.nanotec.es).
- (27) A 10 μ L drop of 10 nM DNA (buffer tris-acetate, pH=7), diluted in 18 MΩ distilled water (ratio 1: 20) was deposited on a flame-annealed gold surface. A voltage of 180 mV was applied to the metal substrate for ~15 min to attract the DNA to the surface electrostatically. Right after deposition, the sample was imaged with an atomic force microscope to check the DNA topography and concentration on the surface (\sim 1–10 molecules in 1 × 1 μ m²), and then the sample was inserted into the STM UHV chamber. A part of the sample, on which DNA was not deposited, was imaged first with the STM for ensuring atomic resolution and verifying the Pt–Ir tip quality (lower resolution is obtained on the part with the deposited DNA, probably due to the water/salt deposit).
- (28) Kotlyar, A. B.; Borovok, N.; Molotsky, T.; Cohen, H.; Shapir, E.; Porath, D. *Advanced Materials* (in press).
- (29) Although we observed contrast inversion on various types of DNA, we present here for the sense of uniformity and simplicity, measurements on homogeneous poly(G)-poly(C) DNA.
- (30) Calev, Y.; Cohen, H.; Cuniberti, G.; Nitzan, A.; Porath, D. *Isr. J. Chem.* **2004**, *44*, 133–143.