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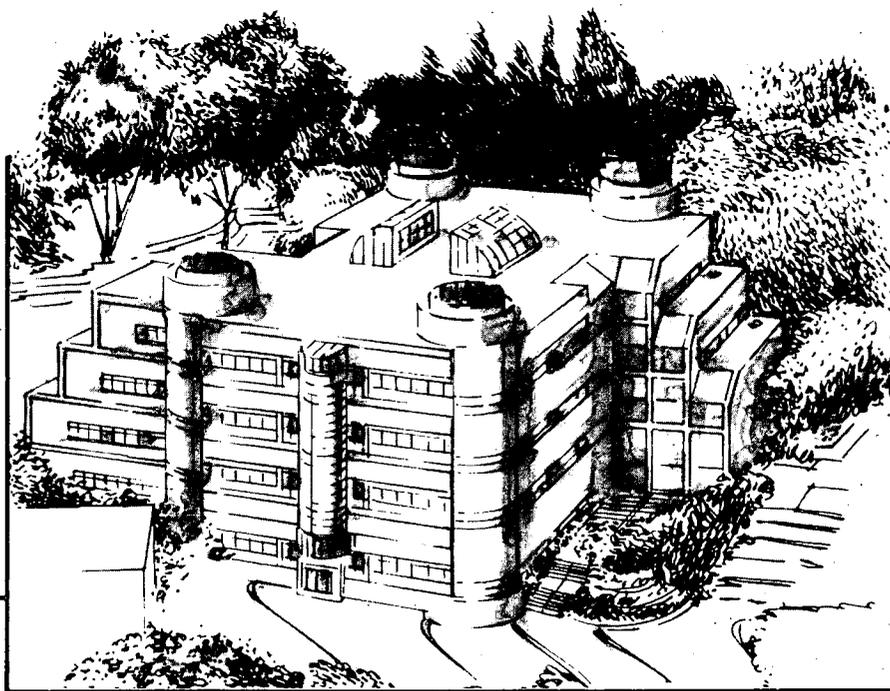
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Scanning Tunneling Microscopy at High Gap Resistances and on Chemically Modified Silicon Surfaces

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ABSTRACT

Scanning Tunneling Microscopy (STM) studies of organic or biological molecules are difficult to reproduce because strong tip-surface interactions can displace weakly-bound material at the surface. We have studied conducting organic monolayers and nucleic acids with our STM instrument at high gap resistance values (100G Ω to 1T Ω). We have succeeded in imaging chemically modified silicon surfaces. It is our desire to use these chemically modified surfaces in conjunction with higher gap values in the future to reliably image DNA.

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1. INTRODUCTION

Repulsive forces exerted by the tip can displace molecules adsorbed on surfaces during STM imaging.^{1,2} These forces are a strong function of tip-adsorbate separation. The repulsive force increases rapidly when the distance separating an atom in the tip from an atom in the adsorbed molecule is less than the sum of the van der Waal radii of the two atoms.³ Stable and reproducible STM images of adsorbed molecules weakly bound to the substrate can only be obtained if the tip-adsorbate separation is always larger than the sum of the van der Waal radii of any pair of tip and adsorbate atoms.

The tip-sample separation during STM imaging depends on the imaging conditions -- the tunnel current, bias voltage, tip shape and composition and the nature of the sample. In topographic (constant current) images the STM electronics adjust the tip height to maintain a preset tunnel current at a given tip-sample bias voltage as the tip is scanned over the surface. The 'gap resistance', the magnitude of the bias voltage divided by the magnitude of the tunnel current, is a useful parameter to describe STM imaging conditions. Tip-sample separation increases monotonically as gap resistance increases. Graphite and metal surfaces are typically imaged at gap resistances of 10-100 M Ω (current \sim 1 nA and bias \sim 10-100 mV), while semiconductor surfaces are typically imaged at 1-10 G Ω (current \sim 0.1-1 nA and bias \sim 1V). The STM contrast mechanism, a complex function of the tip and sample electronic structure, determines the relationship between the gap resistance and the tip-sample separation.

Several organic molecules adsorbed on conductive substrates have been imaged with the STM, including DNA^{4,5}, benzene⁶, and cell membrane

proteins.⁷ These molecules are non-conducting in their liquid and solid phases. The STM contrast mechanism for imaging non-conducting molecules adsorbed on conducting substrates is not well understood. Two limiting cases can be defined. In the 'strong-contrast' limit the adsorbed molecules act as conductors. Electrons can tunnel directly between the tip and the adsorbate, and the tunnel gap width is measured between the tip and the adsorbed molecule. In the 'weak-contrast' limit the adsorbed molecules act as dielectric materials inside the tunnel gap. Electrons tunnel directly between the tip and the substrate, and the tunnel gap width is measured between the tip and the substrate. If the dielectric properties of an adsorbed molecule increase the tunneling probability (positive contrast) a bump will be created in a topographic STM image, while if the adsorbed molecule decreases the tunneling probability (negative contrast), a hole will be created

Tip-adsorbate forces are especially important for imaging in the weak-contrast limit. In this limit the tip-substrate separation may need to be as large or larger than the thickness of the adsorbed molecules to obtain stable STM images.

We believe that in many cases DNA and other adsorbed molecules are imaged in the weak contrast limit. For example, Guckenberger *et al.* report that gap resistances $> 1 \text{ T}\Omega$ ($10^{12} \Omega$, current $< 1 \text{ pA}$) are necessary to image an ordered, two dimensional protein layer with a thickness of 70 \AA .⁷ Further examples are discussed below.

On occasion DNA adsorbed on graphite has been imaged at low gap resistances ($< 100 \text{ M}\Omega$) corresponding to the strong contrast limit^{4,5}, however these observations have been difficult to repeat. These images may be due to

DNA bound strongly to steps, defects or flakes on the graphite. substrate.¹ This could explain both the strong image contrast and the difficulty of reproducing these measurements.

We are working to develop techniques for reproducible imaging of DNA with the STM, including substrates other than graphite and also means of strongly binding the adsorbed DNA molecules to the substrate. In this paper we report on experiments involving silicon substrates. Flat silicon surfaces can be prepared for STM imaging in air. We have results on DNA and RNA imaging in the low-contrast limit. These silicon surfaces can be chemically modified in ways that may allow ionic or covalent binding of DNA to the substrate. Strong binding will reduce the effects of tip-adsorbate forces and may allow imaging in the strong-contrast limit.

2. EXPERIMENTAL

Silicon wafers offer several potential advantages over single crystals of graphite (HOPG) for the imaging of biological material. Silicon is crystalline and is readily available. We can reliably obtain images in air of oxidized silicon surfaces with rms roughness $< 5\text{\AA}$ over areas up to one square micron (Figure 1). Silicon is chemically stable, and we have not observed the types of artifacts, such as structures formed at step edges, that are common with graphite.¹ Silicon is isotropic while graphite has unusual electronic and mechanical properties. Finally, silicon may be chemically modified with organic films to control surface properties and the surfaces can still be imaged after chemical modification.

2.1 Silicon Preparation Procedure

Careful preparation and handling is essential to avoid contamination and degradation of the silicon surface. We used 2" and 4" diameter Si(100) wafers (p-type, boron doped to 15-30 Ω -cm from Uni-Syl) and 4" Si(111) (p-type boron doped to 12-20 Ω -cm from Monsanto) for our studies. In all cases, the silicon wafers were electronic grade quality.

The silicon wafers were cleaned by immersion into a solution of concentrated H_2SO_4 and H_2O_2 at 120°C for 10 minutes in teflon containers. We have determined that 100 ml of 30% H_2O_2 in 1L of H_2SO_4 is sufficient to remove hydrocarbon contamination from the silicon surface.⁸ The wafers were rinsed sequentially in three teflon tanks of flowing de-ionized water at room temperature until the water resistivity was 16 M Ω -cm. The wafers were then stripped of surface oxide by immersion into hydrofluoric acid (HF) at room temperature for 30-300s. The hydrofluoric acid solution was prepared by mixing 49% HF (Aldrich electronic grade, used as received) with H_2O in a ratio of 10:1 (H_2O :HF). The wafers were rinsed again in flowing H_2O until the resistivity was 16 M Ω -cm. A thin surface oxide layer was produced by dipping the silicon wafer into a dilute solution of H_2O_2 at 60°C for approximately 30 sec.

2.2 Nucleic Acid Deposition Procedure

We used three methods to deposit DNA and RNA on the native oxide silicon surface. Our most common technique was to deposit a 20-30 μ l drop on the surface, allow it to remain for 1-5 minutes and then remove it either with inert gas or with an absorbent. On occasion, we allowed the drop to dry

completely on the surface. In such cases, we avoided the salt deposits left from the receding droplet when positioning the STM tip. We observed no differences in image quality between these two methods. The third method of deposition involved applying a ± 1 V bias to an electrode immersed in the droplet while maintaining the silicon substrate at ground. Images taken of samples treated with buffered solutions but without DNA or RNA did not reveal any features. In all cases, the DNA fragments were linear, between 13-1000 bases in length and stored in a buffered solution of 10 mM Tris: 1 mM EDTA at pH 7.5.

3. RESULTS

The observation that DNA binds to glass motivated us to use the oxidized silicon surface as a substrate for DNA imaging.⁹ The majority of our images of DNA revealed aggregates of material on the surface. Less frequently we obtained images showing individual strands of DNA (Figure 2a), although subsequent images indicated that the tip first displaced and then removed the adsorbed material. (Figure 2b).

All of the STM images showing aggregates of DNA were obtained with gap-resistances between 100 M Ω and 100 G Ω . The images were obtained in air using tips cut from Pt/60% Rh/40% wire. In view of our results and those of Guckenberger *et al.*⁷, we modified our electronics to allow operation at very low tunneling currents (<10 pA) in order to decrease the magnitude of the tip-surface interactions. The following examples demonstrate the effects of increasing the tunneling gap resistance.

3.1 Imaging at High Gap Resistance

We studied the effects of high gap resistance on tip-sample interactions using tRNA adsorbed on an oxidized silicon substrate. We exposed a silicon surface to a 10 μ l drop of a buffered solution of 0.5 mg/ml tRNA which we calculated would form a complete monolayer on the surface oxide. One image of the surface was flat and featureless at the tunneling conditions of approximately 900 G Ω (10 pA and 8.9 V, sample negative). Subsequently, we scanned an inset image at a reduced gap resistance of 300 G Ω (10 pA at 3V, sample negative). Finally, a second large image taken at the original high gap resistance revealed a hole in the center of the image. The hole corresponds both in position and dimensions to the area scanned at lower gap resistance (Figure 3). The depth of the hole is 35-40 \AA which is comparable to the dimensions of isolated tRNA molecules.¹⁰ We explain this observation by assuming that the tRNA has formed a nearly complete monolayer at the surface and that imaging at a 300 G Ω gap resistance removed the molecules from the imaged area. Control experiments with the native silicon oxide surface treated with the buffered solutions but without tRNA were not changed by imaging at 300G Ω . It appears then that gap resistances of less than 500 G Ω may be sufficient to remove weakly-bound tRNA molecules from the native oxide.

We have also studied polymerized organic films lifted on to HOPG single crystals using STM which provide additional evidence of tip-surface interactions. Figure 4a shows an STM image of such a film with a nominal thickness of 40 \AA taken at 1 T Ω (corresponding to 10pA at 10V) which shows the fibrous structure of the organic film and a large diagonal step in the

underlying graphite substrate. The identical area was subsequently imaged at a lower gap resistance of 13 G Ω is shown in figure 4b. The graphite step is still present but the film is no longer visible. Returning to higher gap resistance did not recover the organic film structure. Subsequently, we enlarged the scan area at high gap resistance and found a rectangular hole in the film surrounded with debris. We have reproduced this damage on several different organic film samples. Our results indicate that tip sample interactions are critically dependent on the tunneling gap resistance. The onset of strong repulsive interactions sets a lower limit on the tip-sample separation for imaging non-conducting or poorly-conducting materials.

3.2 Chemical Modification of Silicon Substrates

We believe that we can increase the resistance of the adsorbate molecules to displacement by the tip by increasing the adsorbate-substrate binding affinity. We can modify the interfacial properties of oxidized silicon wafers using alkylsiloxanes. These are organic molecules with an activated silicon atom at one end and a functional group at the other end separated by several carbon atoms (Figure 5). The choice of functional groups allow control over the chemical properties of the resulting surface. Oxidized silicon surfaces modified with alkylsiloxanes have also been characterized with a variety of surface science techniques.^{11,12} We have prepared silylated silicon surfaces by immersing clean silicon wafers into a solution of freshly distilled anhydrous ethyl ether containing the alkylsiloxane for 1-24 hours.¹³ The surfaces are rinsed several times with clean, distilled ether and dried with

flowing nitrogen gas. We have studied the resulting surfaces with XPS and contact angle measurements to verify that the film is present on the surface.

We have prepared silicon surfaces using alkylsiloxanes with amine, thiol and hydrocarbon head groups. We have studied these surfaces with STM and although no molecular-level structure has yet been observed, the films were flat with an RMS roughness of 5 Å. Thus, the chemically modified silicon surface are sufficiently flat to allow good contrast against larger adsorbates. We are presently determining the optimal conditions for monomeric DNA adsorption. Our preliminary results indicate that in the case of the aminopropylsiloxane films, the amine surface has a much lower pK_a compared to free amines in solution. We have demonstrated that we can manipulate the pH of the solutions to protonate the surface amines ($-NH_2 \rightarrow -NH_3^+$) and follow the surface properties with measurements of contact angle.¹⁴ It is our goal to generate a protonated amine surface which will form a salt bridge with the negatively charged phosphodiester backbone of nucleic acids. We are also working on the development of chemistry which will allow the covalent attachment of DNA and RNA to the surface.

4. CONCLUSIONS

In summary, we have modified our STM to operate at gap resistance values as high as 1 TΩ in order to decrease the tip-surface interactions with adsorbates. We believe that tip interaction with the adsorbate is a major reason for the lack of reproducibility in the imaging of biological samples. We have presented several experimental results where the van der Waal dimensions of molecules can be correlated with the minimum gap resistance

allowed for reliable imaging of the adsorbate. Finally, we are also working on methods to attach biomolecules to the surface more strongly with the use of covalently bound films on silicon.

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Figure Captions

1. 500 Å x 500 Å image of the silicon oxide surface imaged in air. The sample was cut from a Si(111) wafer and imaged at a gap resistance of 137 GΩ (20 pA and 2.75 V bias, sample negative). The image appears flat within the RMS noise of the electronic feedback (approximately 5 Å).
- 2a. 800Å x 800Å image of several DNA oligomers. The DNA was deposited from a 1 ml sessile drop of a 4 mg/ml solution which consisted of fragments of DNA with an average length of 434 base pairs. An electrode was immersed in the DNA drop on the surface and a voltage difference of +/- 1V applied maintaining the silicon substrate at ground. The voltage difference was maintained for 2 minutes after which the silicon surface was rinsed several times with a 95% ethanol solution. The image was obtained at a gap resistance of 277 GΩ. We interpret the bright streaks running diagonally across the image as strands of DNA.
- 2b. An image of the same area taken immediately after the image in figure 2a. The positions of the streaks have changed from the previous image indicating that 267 GΩ is not a sufficiently large gap resistance to suppress tip-surface interaction. The image taken after this showed a flat silicon surface with no features. We interpret this as indicating that the scanning tip first displaced and then removed the adsorbed DNA at these gap values.
3. 6920 Å x 5800 Å image of a hole in a monolayer of tRNA adsorbed to the native oxide silicon surface. This image was taken at a gap resistance of 896 GΩ (10pA and 8.96V sample negative). The hole was formed by scanning an area 3000 Å x 3000 Å at a gap resistance of 300 GΩ (10 pA at 3V sample negative) at a scan speed of 9160 Å/s. The tRNA was deposited from a sessile 10µl drop of 0.5µg/ml solution of tRNA (Tris-HCl buffered pH 8.0, 10mM MgCl₂, 150 mM NaCl).
- 4a. 27800 Å x 34200 Å image of a polymerized organic film on graphite. An underlying step is visible running diagonally

across the image. The gap resistance for the image was $1 \text{ T}\Omega$ (10 pA at 10V, sample negative) and the tip velocity was 42780 \AA/s . The thickness of the polymer is approximately 40 \AA .

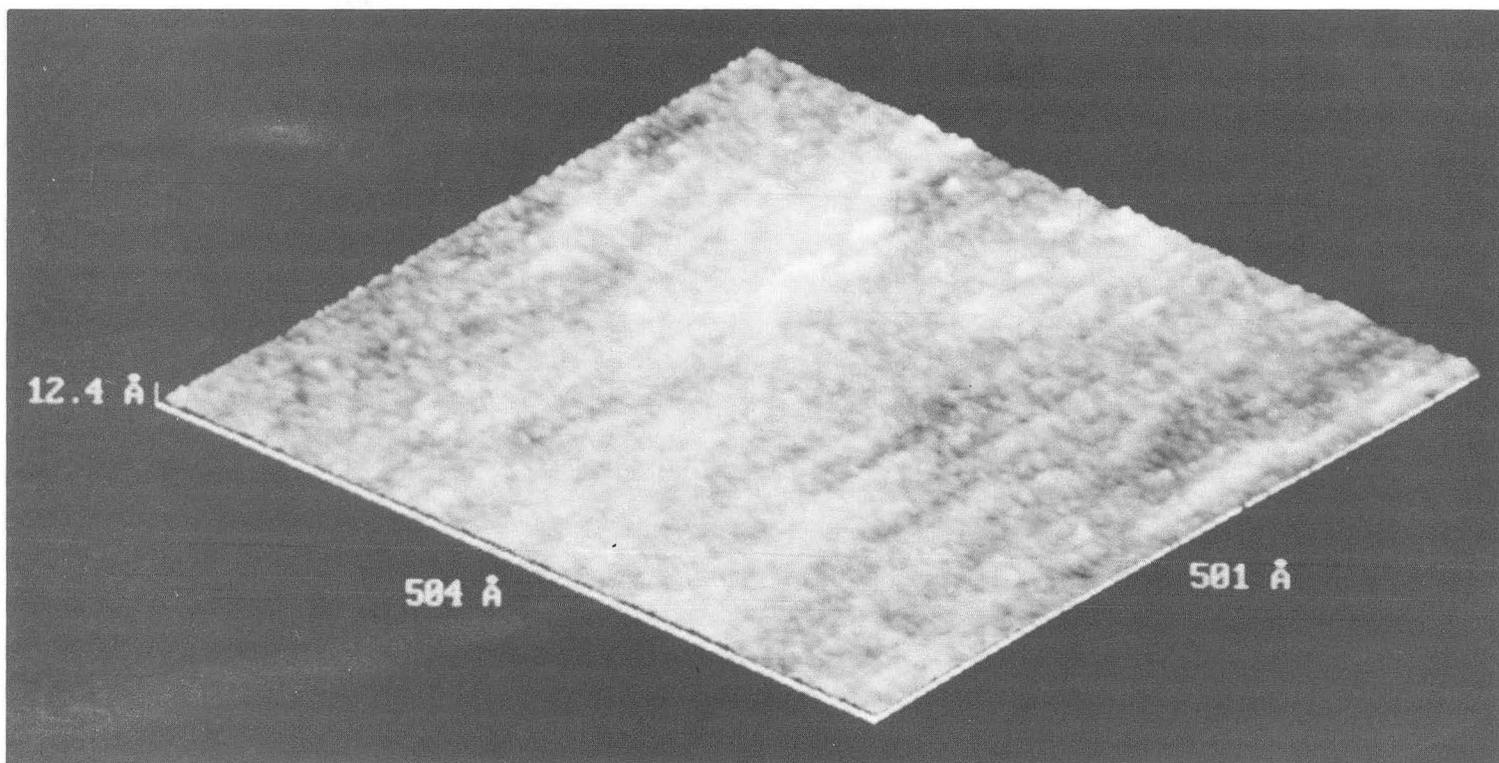
- 4b. The same area as before imaged at lower gap resistance values of $13.0 \text{ G}\Omega$ (10pA at 130mV, sample negative). The fibrous structure of the film evident in Fig. 4a has been removed leaving only the flat graphite step. Returning to higher gap resistance does not recover the film structure.
5. Above: Schematic representation of alkylsilane (Aminopropylsiloxane) used to modify silicon surface oxides.

Below: We can change the chemical and physical properties of the silicon surfaces and bind biomolecules more strongly by chemical modification with alkylsiloxanes. We show the process schematically for amines, thiols and hydrocarbon groups. The diversity in the choice of alkylsiloxane monomers may allow for the construction of surfaces with ionic, covalent or intercolative binding ability.

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14. M. N. Murray, D. F. Ogletree and M. B. Salmeron, to be published.

Figure 1



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Figure 2a

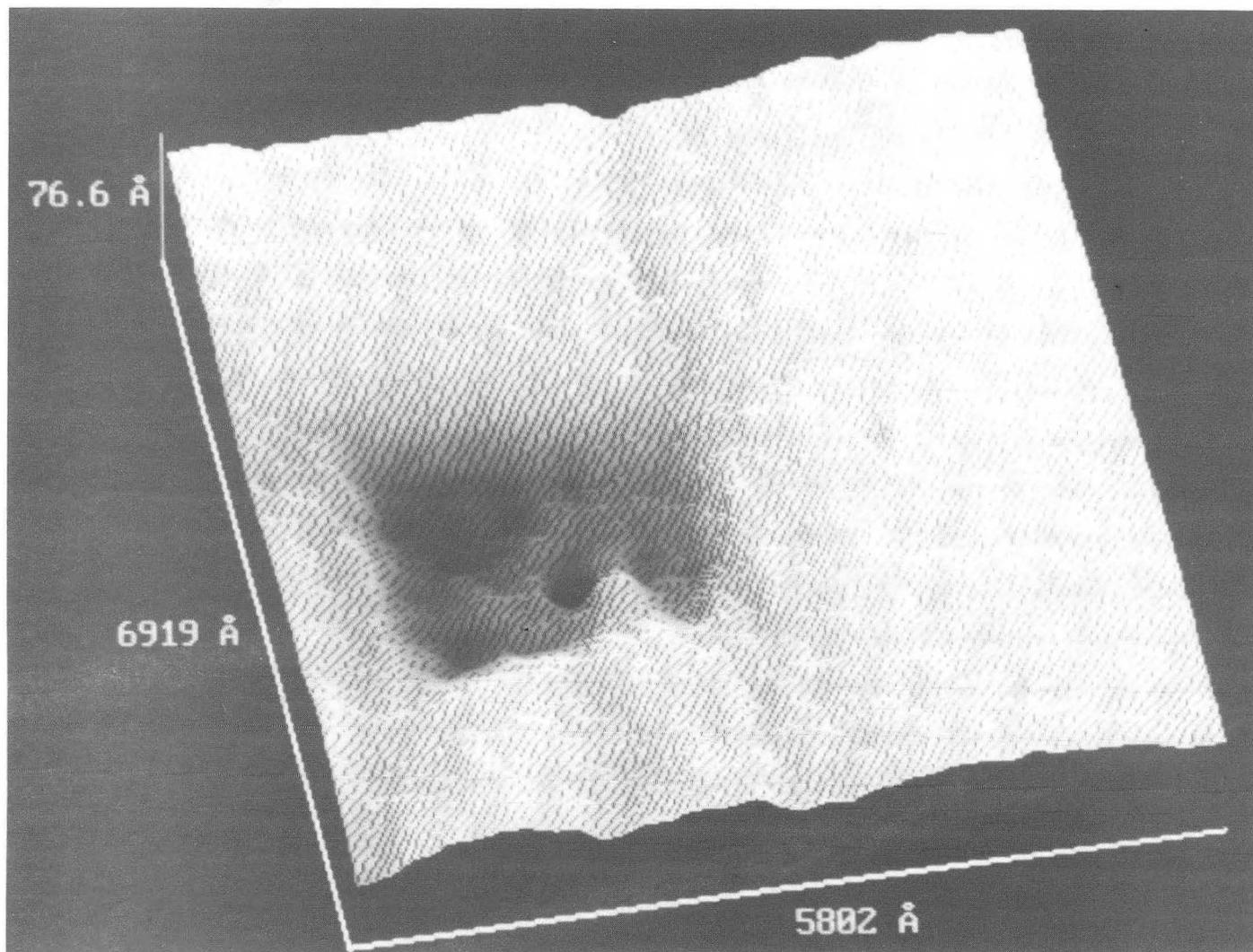


Figure 2b



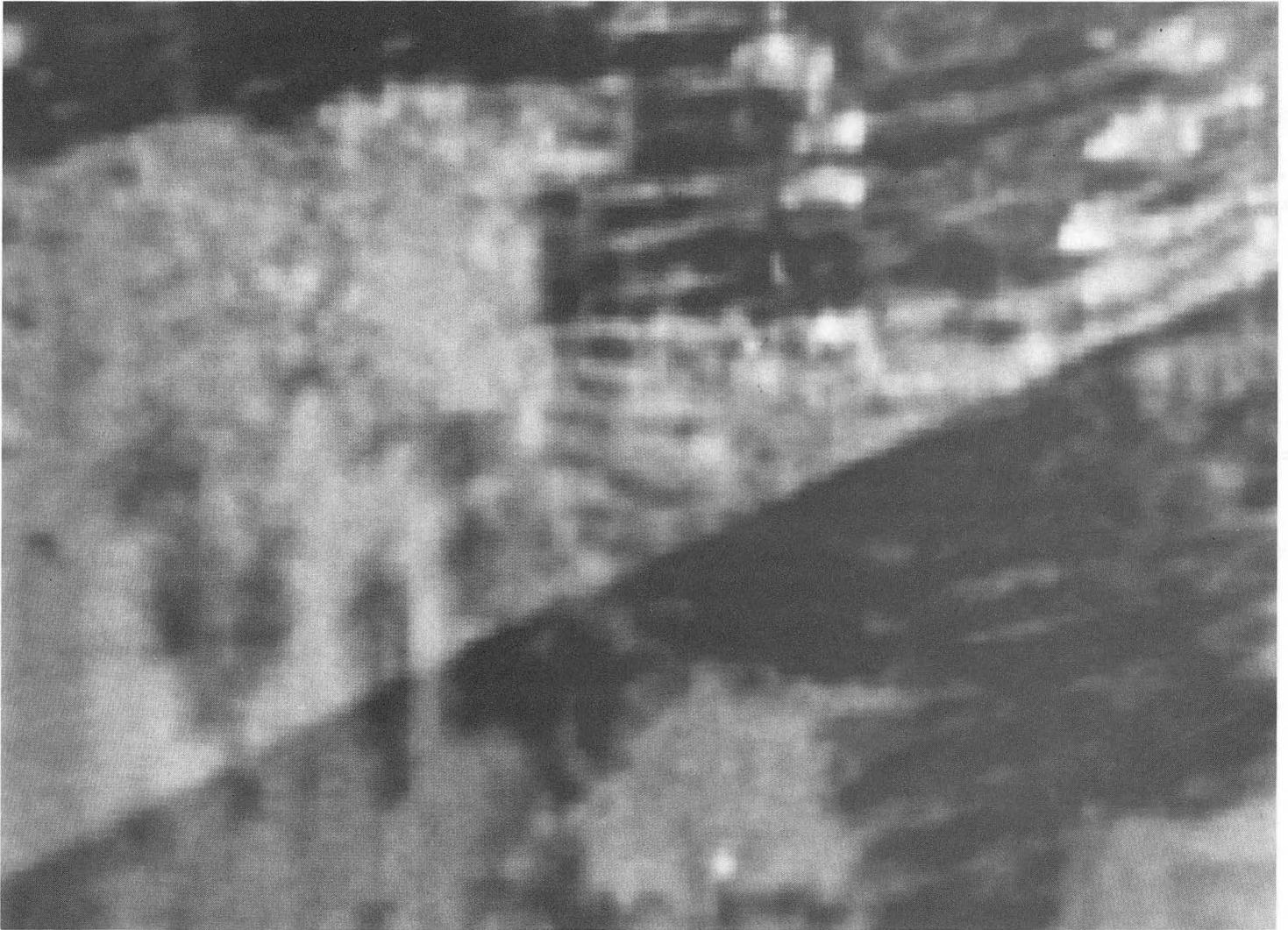
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Figure 3



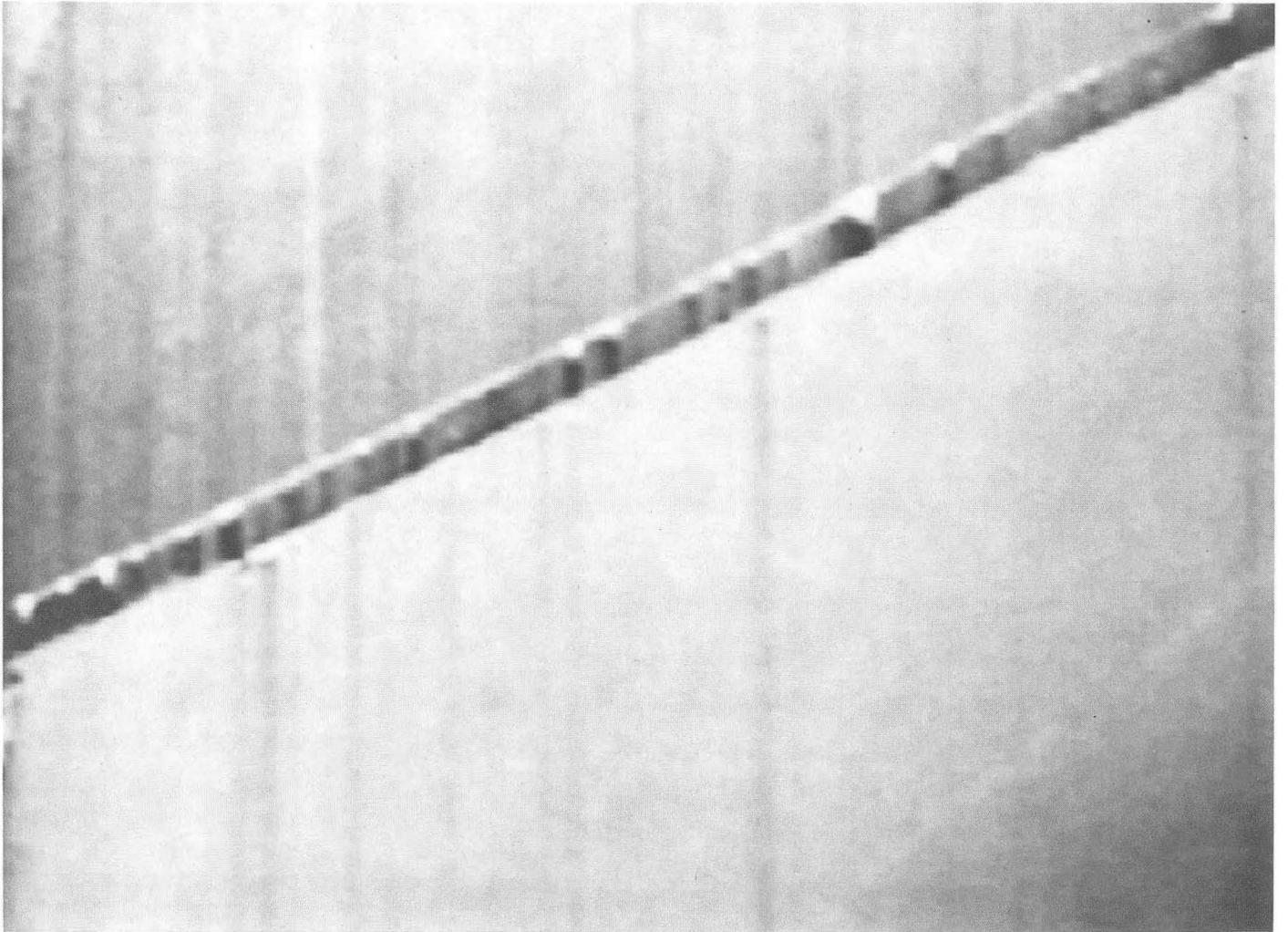
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Figure 4a



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Figure 4b



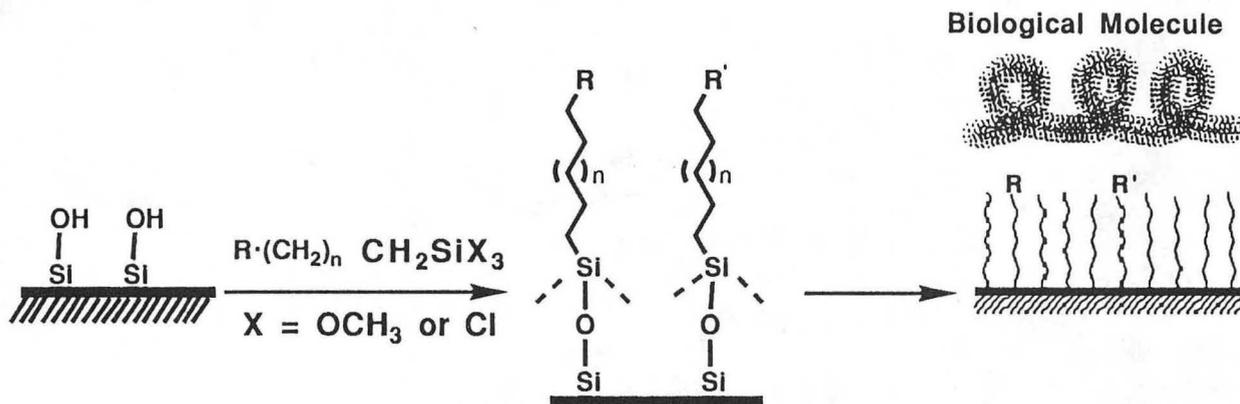
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Figure 5

Trimethoxyaminopropylsiloxane



Chemical Modification of Silicon Surface Oxide



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