Lawrence Berkeley National Laboratory
LBL Publications

Title

DIRECT OBSERVATION OF NATIVE DNA STRUCTURES WITH THE SCANNING TUNNELING MICROSCOPE

Permalink

https://escholarship.org/uc/item/2hc9h7b8

Authors

Beebe, T.P.
Wilson, T.E.
Ogletree, D.F.

Publication Date

1988-10-01

eScholarship.org Powered by the California Digital Library
University of California

LBL-26201 c.2
Preprint

Center for Advanced Materials

CAM

Submitted to Science

Direct Observation of Native DNA Structures with the Scanning Tunneling Microscope

T.P. Beebe, Jr., T.E. Wilson,
D.F. Ogletree, J.E. Katz,
R. Balhorn, M.B. Salmeron, and
W.J. Siekhaus

October 1988

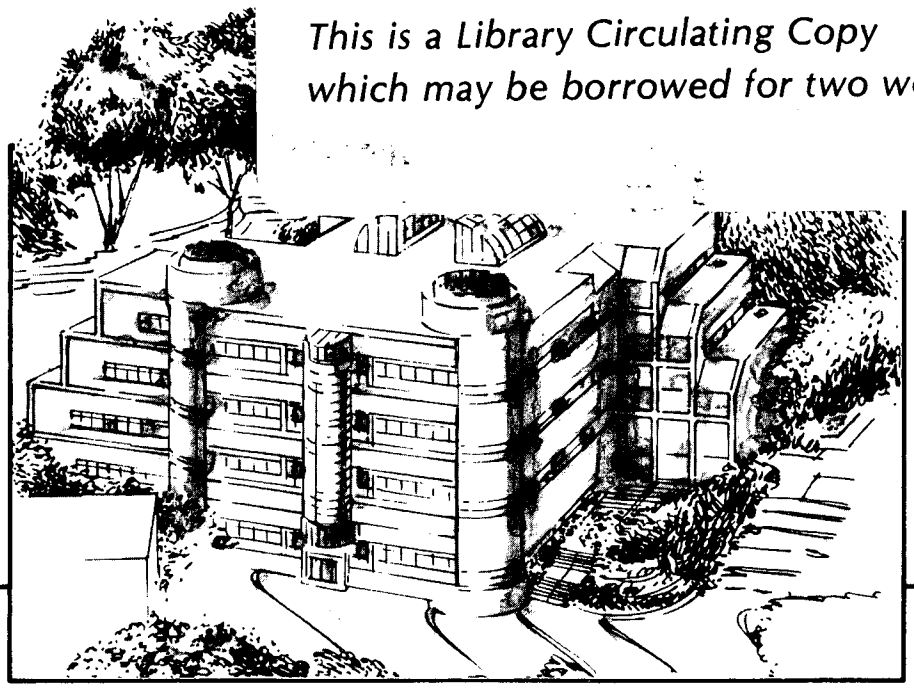
RECEIVED
LAWRENCE
BERKELEY LABORATORY

FEB 15 1989

LIBRARY AND
DOCUMENTS SECTION

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks.



Materials and Chemical Sciences Division

Lawrence Berkeley Laboratory • University of California

ONE CYCLOTRON ROAD, BERKELEY, CA 94720 • (415) 486-4755

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

LBL-26201 c.2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Direct Observation of Native DNA Structures with the Scanning Tunneling Microscope

Thomas P. Beebe, Jr.,[1] Troy E. Wilson,[1] D. Frank Ogletree,[2]
Joseph E. Katz,[3] Rod Balhorn,[4]
Miquel B. Salmeron,[2] and Wigbert J. Siekhaus[1]

key words: DNA, scanning tunneling microscopy, STM

[1] Lawrence Livermore National Laboratory, L-357, Department of Chemistry, Livermore, CA 94550

[2] Lawrence Berkeley Laboratory, Materials and Chemical Sciences Division, Center for Advanced Materials, 1 Cyclotron Road, MS 66-212, Berkeley, CA 94720

[3] Lawrence Berkeley Laboratory, Engineering Division, 1 Cyclotron Road, MS 70A-3363, Berkeley, CA 94720

[4] Lawrence Livermore National Laboratory, L-452, Biomedical Sciences Division, Livermore, CA 94550

October 1988

ABSTRACT

The spiraling structure of native double stranded DNA deposited on graphite has been imaged in air with the Scanning Tunneling Microscope. The resolution achieved by our STM allowed us to distinguish the major and minor grooves. The pitch of the helix varied between 27 and 63 Å in the images shown. This shows that the STM can be useful for structural studies of a large variety of biomolecules that cannot easily be crystallized.

In this communication we present the first high resolution observations of deoxyribose nucleic acid (DNA) structures with the scanning tunneling microscope (STM) in native (i.e. naked or non metal-shadowed) specimens. Our high resolution images show that the double helix structure of DNA can be resolved. In addition, the distances between the grooves have been measured. This is the first time that such resolution has been achieved on an isolated strand of native DNA with this or any other technique [1]. Our results suggest the possibility that even higher resolution can be obtained which will allow the individual nucleotide base structures to be resolved.

Scanning tunneling microscopy (STM) results are now emerging on a large variety of surfaces other than the semiconductor and metal surfaces used in the initial studies. Particularly relevant to the biological sciences are those investigations in which images of organic, inorganic, and biological molecules have been presented, both in air and under a variety of other media. These include, among others, bacteriophage virus particles [2], metal-shadowed recA-DNA complexes [3,4], native closed circular DNA [4], small organic molecules such as benzene [5] and copper phthalocyanine [6,7], conducting polymers [8], films of liquid crystals [9], Langmuir-Blodgett films [10,11], and freeze-fracture metal replicas of biomembranes [12]. These early studies are promising because they demonstrate that the power of the scanning tunneling microscopy technique (simplicity of operation, low cost, sub-Angstrom x,y and z resolution, and imaging in a wide variety of environments including air or liquids) can be brought to bear on structural questions in the biological field, even if the exact nature of the electron energy levels of the organic molecule that participate in the tunneling process is not known or understood. Since the STM offers unprecedented spatial resolution in three dimensions and does not require the harsh sample preparation conditions needed for most conventional electron microscopy techniques, we believe that significant progress can be made toward understanding the structure and function of DNA in its natural, hydrated state.

We have constructed a scanning tunneling microscope based on an earlier design [13] using a micrometer driven differential spring mechanism

for sample movement and a tubular piezo rather than a tripod to scan the tip. Scanning and feedback control electronics, developed in our laboratory, are interfaced to a computer which controls the real- time acquisition and storage of STM data. The particular conditions of tunneling are typically 150 mV sample bias (either polarity) and a few nA tunneling current. No difference in the images was observed as a function of these parameters. Solutions of calf thymus DNA were prepared as described in [14]. A droplet of the aqueous solution (1 mg DNA/ml H_2O in 10 mM KCl) was allowed to evaporate in air on a freshly cleaved, highly ordered pyrolytic graphite (HOPG) substrate [15] which provides a conductive surface with atomically flat crystal planes over thousands of Angstroms. We employed mechanically cut tips consisting of a 60% Pt, 40% Rh alloy. In the images presented here, tunneling was initiated immediately after the last amounts of water were observed to evaporate. Thus, the DNA was not subjected to particularly harsh or intricate sample preparation procedures. Sample blanks consisting of 10 mM KCl solutions subjected to identical treatments, but not containing DNA, showed no topographic structure in the STM images other than the expected atomic features and occasional steps which are characteristic of cleaved graphite surfaces.

Some of the problems encountered with DNA prepared in this way included clumping of the DNA into unrecognizable large aggregates which did not give stable tunneling conditions, and the other extreme, that of not being able to find any DNA molecules within the scanning range of our microscope (our largest scans are 0.5 μm$\times$0.5 μm). The complete evaporation of the DNA solution commonly left a 4 mm area on the graphite surface consisting of a series of concentric rings due to the evaporation process and the salts which the shrinking droplet left behind. In the center of this area there was usually a 1 mm spot in which the last amounts of salt precipitated out of solution. This region was avoided due to the unstable tunneling conditions caused by the thick layer of non-conducting salt.

Figure 1a shows an image constructed from the topographic contours followed by the STM tip as it traversed the DNA adsorbed on the graphite, while the feedback control electronics maintained a constant tunneling current. The intensity of the gray scale is proportional to the height of the tip,

white being high, black being low. This image is typical of our large area images, in that it shows a DNA duplex (i.e. double stranded DNA molecule) which makes many convolutions on the surface, and consists both of segments which are isolated from the rest of the duplex, as well as segments in which there are overlapping and apparently tangled DNA duplexes. The periodic bumps along the duplex occur with a range of spatial periodicities from 27 to 50 Å and correspond to the pitch of the spiraling DNA helix. The observed DNA structures were stable and reproducible from scan to scan.

Figures 1b and 2 are magnified views of different regions on the sample. In Figure 1b, two DNA duplexes are observed lying nearly parallel on the substrate; the pitch periodicities range from 28 to 50 Å, with an average over 9 measurements along the duplex corresponding to approximately 36 Å. The appearance of a twisted ladder is seen here, although no alternation in groove size is apparent as would be expected if the major and minor grooves were resolved. Figure 2, taken from another area on the sample, shows a spiraling right-handed DNA duplex in greater detail. Four measurements of the DNA pitch taken along this particular length range from 46 to 52 Å, with an average of 49 Å. Although the apparent width of the DNA seems large in this image (approximately 60 Å), this apparent width is really a result of the finite size of the tip. This problem becomes more pronounced for structures with a large relief from the substrate, as tunneling from the side of the tip is possible. The apparent width is thus a convolution of the actual DNA structure with that of the tip. The height above the substrate (i.e. change in the z coordinate) is not affected in the same way, and this value is typically 20 to 30 Å, close to the expected value.

Although this and the previous image (i.e. figures 1 and 2), obtained from different regions of the sample, were acquired only minutes apart in time, the pitch periodicity differs by 37 %. The hydration state of the DNA is only one factor which determines its molecular conformation. Since the conformation varies greatly even for samples which have had nearly identical times for dehydration to occur, it is likely that other forces, perhaps surface-molecule interactions, are responsible for the structures which we observe. It is also possible that our images are a snapshot of the dynamic

molecular conformation at the instant of water evaporation.

Figure 3 is an image in which especially high resolution was achieved. We obtained several images of similar quality which will be published later in a longer paper. The DNA duplex enters the area of figure 3 in the upper right corner of the image, makes a loop and crosses over itself in the upper left corner of the image, and leaves the image in the upper right corner (there is another structure below this, possibly a DNA fragment, about which we will not comment further). The raw image is shown in figure 3a; figure 3b has been processed (see caption), and a schematic conception of the image is shown in figure 3c. In figure 3 there is a long-short alternation in the spacing of the ridges in the DNA duplex. These ridges are due to the phosphodiester backbone of DNA which is composed of alternating deoxyribose sugar and phosphate groups. The alternation observed may be the manifestation of the major and minor grooves. In figure 2, for example, the resolution was only great enough to observe the major groove, and each bump is due to the periodic double-stranded helix separated by the major grooves. In figures 3a and b, the additional structure due to the major-minor groove alternation is seen. The areas between the strands are regions where individual base pairs would be seen if our resolution was great enough. With this interpretation, the distance of one major-minor pair is approximately 63 Å across the bottom duplex of the image, and 49 Å across the top duplex of the image. On average, this corresponds approximately to a 55% length expansion compared to the crystalline state. There is evidence that DNA may undergo length expansions by 25 to 50% when intercalated with ionic species in solution [16]. The DNA molecules have been subjected to the combined forces of the surface, dehydration, and possibly intercalation with ionic species, thus we are not surprised that the pitch periodicities vary as they do here.

At an even greater level of inspection, localized bright spots are observed along the DNA backbone in figure 3a. At present, we cannot conclude whether these spots are due to noise, or to the sugar and phosphate groups.

It is of interest to note that these images were obtained from an area swept by the shrinking droplet after the aqueous solution evaporated. We

speculate that the observed DNA molecules were trapped on defects in the graphite substrate and then left behind by the receding water droplet. The trapping of the DNA and the role of the K and Cl ions and surface defects may be crucial elements of the process which holds the DNA to the surface. We postulate that these combined effects may lend significant structural stability to the adsorbed DNA molecules.

The ability to manipulate semiconductor and metal atoms with the STM has been demonstrated [17-20], as has the ability to fragment, attach, and remove simple organic molecules on the surface of graphite [21]. Given our initial success in obtaining these images with such detail, we believe that it may be possible to significantly advance the field of molecular biology, using the STM as both a real-space sequencer and micro-manipulator. Using the various spectroscopic capabilities of the STM, we are investigating whether spectroscopic differences are observed at different points along the DNA molecule. This would allow a distinction between different base pairs. Once it becomes possible to identify individual bases, it should eventually be feasible to cut and manipulate the DNA at any chosen location with complete selectivity. We believe that the promise of the STM for performing such operations on genetic material in situ should make this an important technique in genetic and biophysical research. We anticipate that the revolution which the STM and related techniques have caused in the field of surface science will shortly be followed by an even larger revolution in molecular biology and biophysical research.

REFERENCES AND NOTES

1. Diffraction techniques which employ crystalline samples can in principle achieve such high resolution. However, the STM is a real-space technique which can observe isolated molecules, and is not limited by the need for crystalline samples. This capability may be of paramount importance to biochemical studies.

2. A.M. Baro, R. Miranda, J. Alaman, N. Garcia, G. Binnig, H. Rohrer, Ch. Gerber and J.L. Carrascosa, *Nature*, **315**, 253 (1985).

3. M. Amrein, A. Stasiak, H. Gross, E. Stoll and G. Travaglini, *Science*, **240**, 514 (1988).

4. G. Travaglini, H. Rohrer, M. Amrein and H. Gross, *Surf. Sci.*, **181**, 380 (1987).

5. H. Ohtani, R.J. Wilson, S. Chiang and C.M. Mate, *Phys. Rev. Lett.*, **60**, 2398 (1988).

6. J.K. Gimzewski, E. Stoll and R.R. Schlitter, *Surf. Sci.*, **181**, 267 (1987).

7. P.H. Lippel and R.J. Wilson, *Proceedings of the Third International Conference on STM*, Oxford, July, 1988, in press.

8. T. Sleator and R. Tycko, *Phys. Rev. Lett.*, **60**, 1418 (1988).

9. J.E. Frommer and J.S. Foster, *Nature*, **333**, 542 (1988).

10. L. Eng, H.R. Hidber, L. Rosenthaler, U. Staufer, R. Wiesendanger, H.J. Guntherodt and L. Tamm, *J. Vac. Sci. Technol.*, **A6**, 358 (1988).

11. C.A. Lang, J.K.H. Horber, T.W. Hansch, W.M. Heckl and H. Mohwald, *J. Vac. Sci. Technol.*, **A6**, 368 (1988).

12. J.A.N. Zasadzinski, J. Schneir, J. Gurley, V. Elings and P.K. Hansma, *Science*, **239**, 1013 (1988).

13. M. Salmeron, D.S. Kaufman, B. Marchon and S. Ferrer, *Appl. Surf. Sci.*, **28**, 279 (1987). A modified version of this microscope is commercially available from McAllister Technical Services, Berkeley, CA.

14. Highly polymerized calf thymus DNA (Worthington Biochemical Corp., Freehold, NJ) was dissolved overnight in aqueous 10 mM KCl at a concentration of 4.5 mg/ml. Aliquots of this stock solution were subsequently diluted with 10 mM KCl to a working concentration of 1 mg/ml.

15. HOPG was kindly provided by A.W. Moore of Union Carbide.

16. W.K. Olson, "The Flexible DNA Double Helix: Theoretical Considerations", in *Nucleic Acids: Geometry and Dynamics*, ed. R. Sarma, (Pergamon Press: New York, 1980), p. 383.

17. U. Staufer, R. Wiesendanger, L. Eng, L. Rosenthaler, H.R. Hidber, H.J. Guntherodt and N. Garcia, *J. Vac. Sci. Technol.*, **A6**, 537 (1988).

18. E.E. Ehrichs, R.M. Silver and A.L. deLozanne, *J. Vac. Sci. Technol.*, **A6**, 540 (1988).

19. B. Marchon, M. Salmeron and W. Siekhaus, *Surface and Coatings Technloogy*, in press.

20. J.A. Golovchenko, *Science*, *232*, 48 (1986).

21. J.S. Foster, J.E. Frommer and P.C. Arnett,*Nature*, **331**, 324 (1988).

22. This work was supported by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48, and by an LLNL Institutional Research & Development grant. This work was also supported by the Lawrence Berkeley Laboratory through the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials

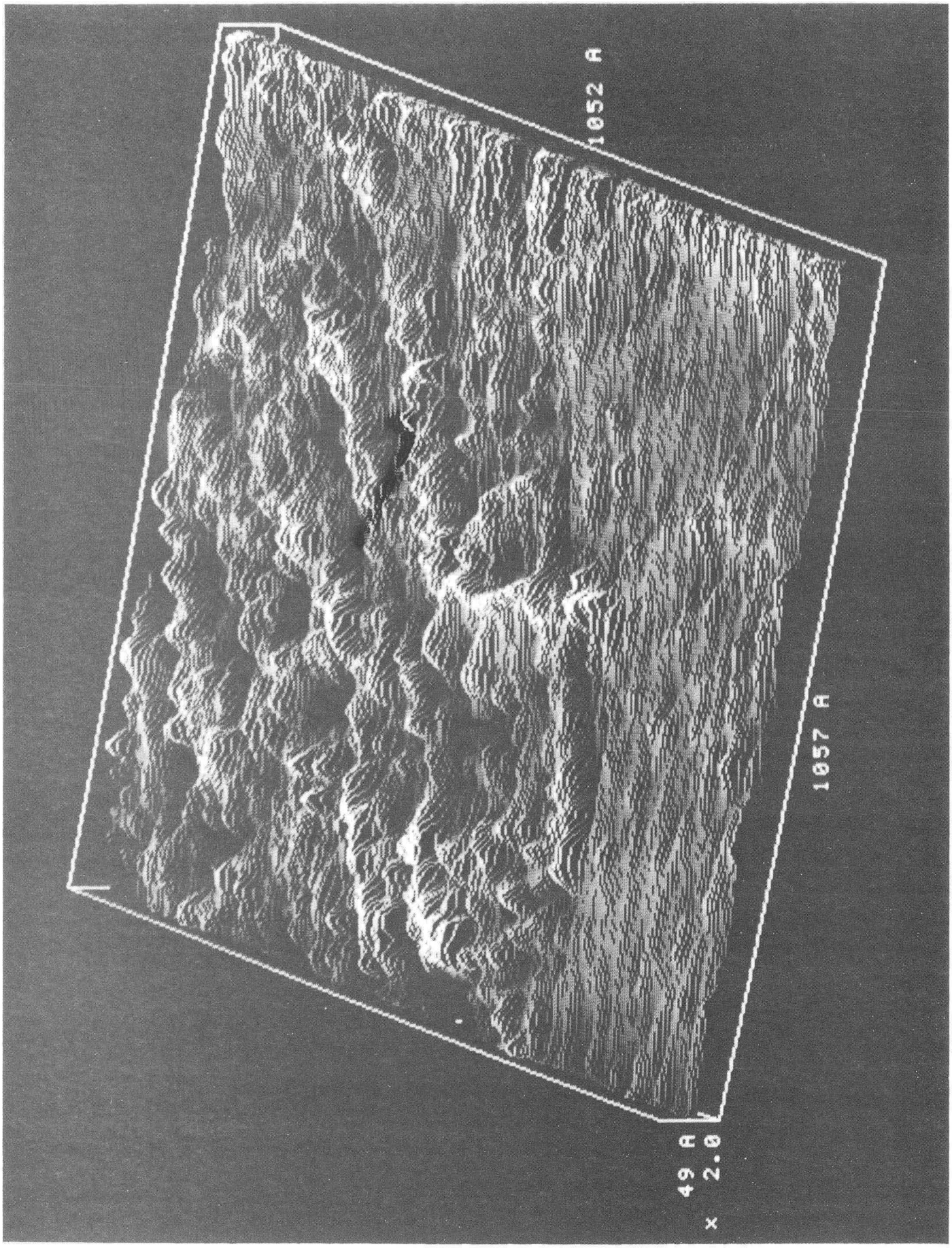
Division of the US Department of Energy under contract number DE-AC03-76SF00098. We wish to thank Dr. John Holzrichter of LLNL for support and insistent encouragement.

FIGURE CAPTIONS

Figure 1. DNA deposited on graphite, imaged in air with the scanning tunneling microscope. All images were obtained at constant tunnel current (i.e. they are "topographic images"). 1a.) area 1050 x 1050 Å, with total height of 47 Å; sample bias -155 mV; current setpoint 0.9 nA; gap resistance 172 MOhm; acquisition time 210 seconds, corresponding to a tip velocity of 1300 Å/sec. Post acquisition image processing consisted of digital bandpass filtering with the removal of all Fourier components above 9.72×10^{-2} $Å^{-1}$ and below 2.43×10^{-3} $Å^{-1}$. Variation of these values within reasonable ranges caused no significant changes in the images. The image is presented in a projected three dimensional format with gray scale, as viewed from a perspective 45 degrees above the plane, and 20 degrees clockwise in the plane. 1b.) same conditions as 1a, showing magnified view of center of 1a, area 260×260 Å, total height range 48 Å; viewing perspective is 45 degrees above plane and 30 degrees counterclockwise in plane.

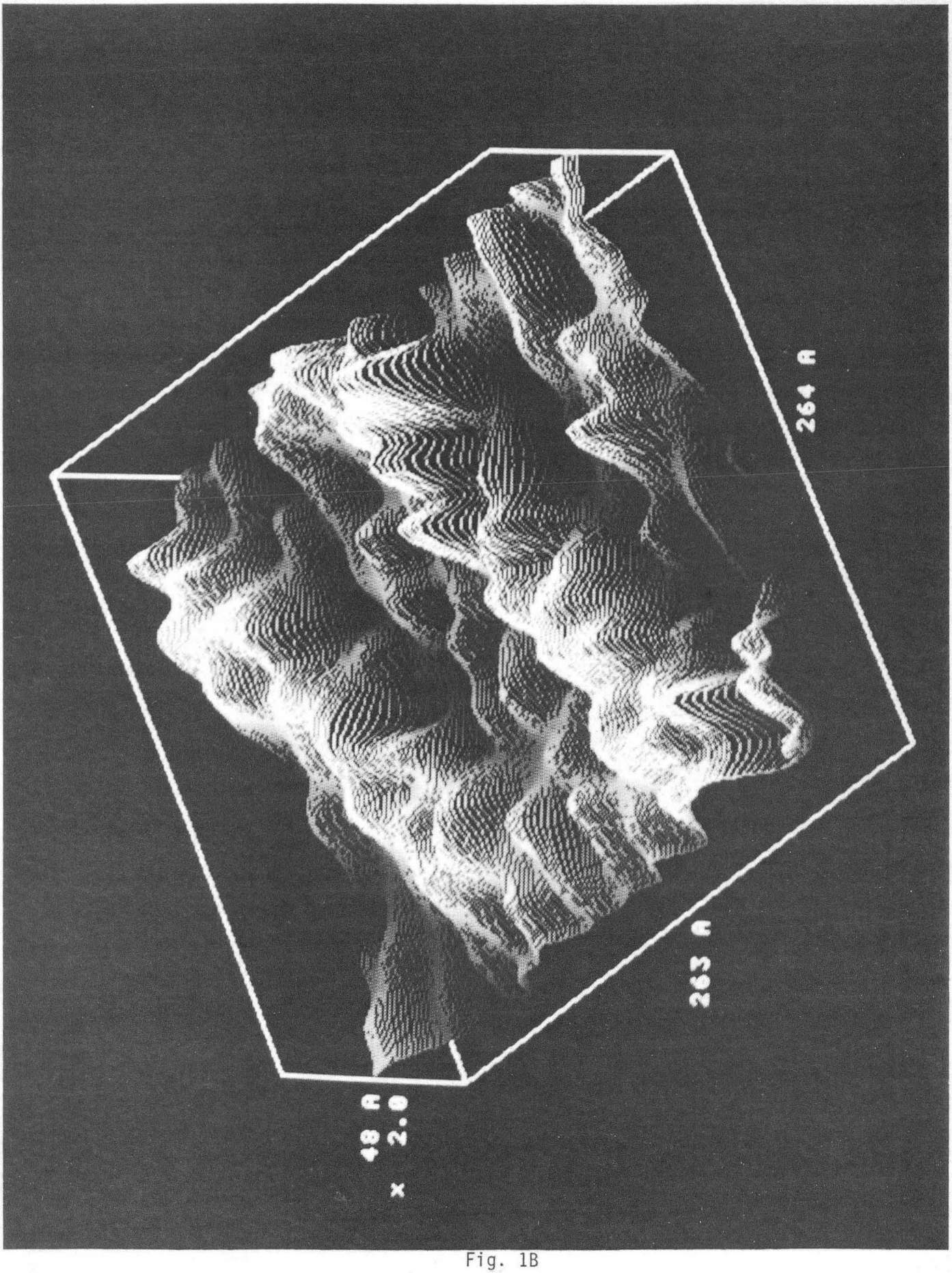
Figure 2. DNA deposited on graphite, imaged in air with the scanning tunneling microscope; image area 340×340 Å, total height range 29 Å; sample bias -155 mV; current setpoint 0.8 nA; gap resistance 194 MOhm; viewing perspective is 45 degrees above plane and 20 degrees clockwise in plane.

Figure 3. DNA deposited on graphite, imaged in air with the scanning tunneling microscope. Topographic image of area 400×400 Å; total height range 132 Å; sample bias -97 mV; current setpoint 3.3 nA; gap resistance 29 MOhm; acquisition time 42 seconds, corresponding to a tip velocity of 2500 Å/sec. 3a.) raw image. 3b.) post acquisition image processing consisted of 9 point two-dimensional smoothing applied once, followed by simulated light source shading from a point 15 degrees above the plane. 3c.) schematic conception of the DNA structure seen in figures 3a and 3b.



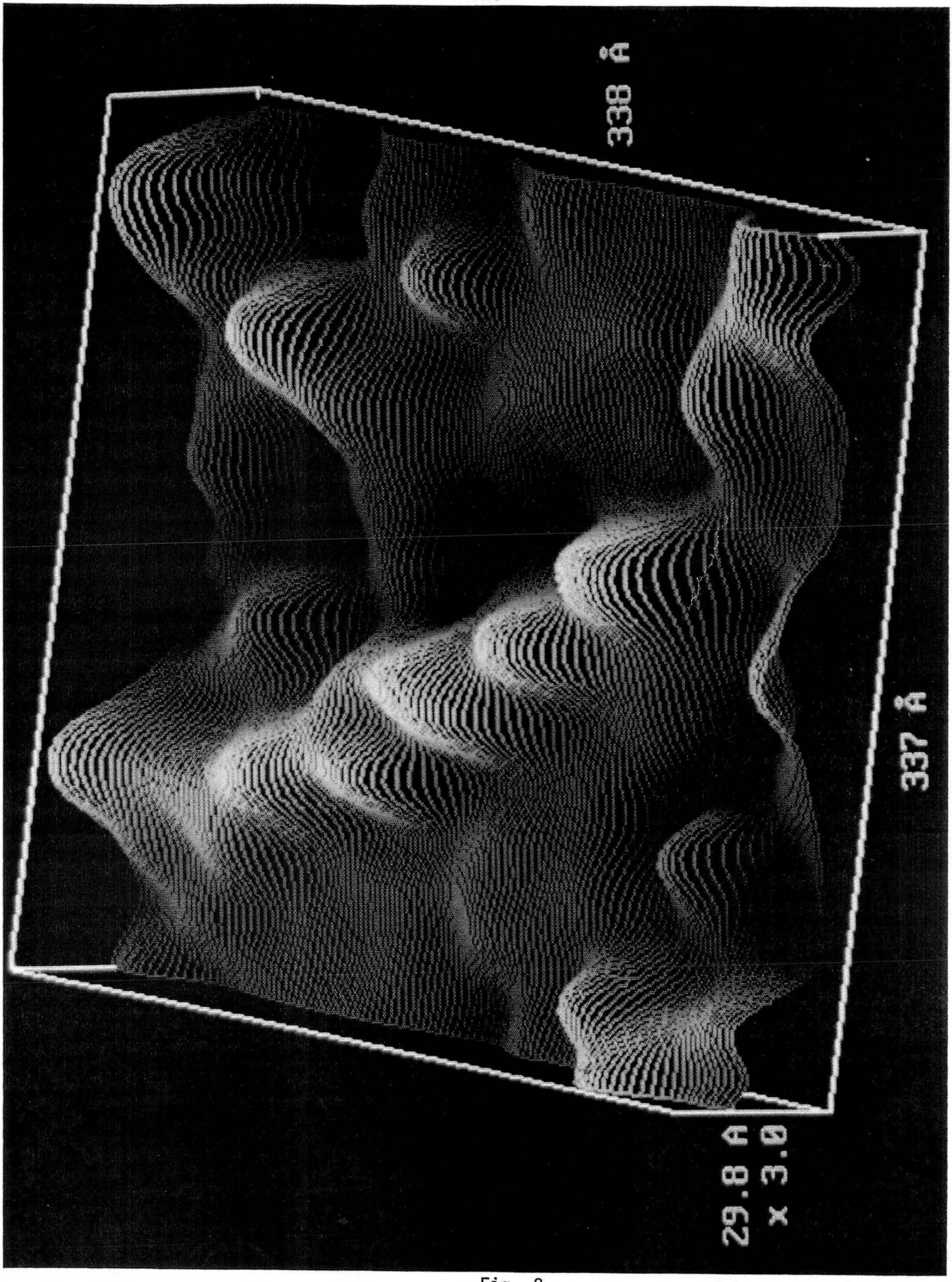
XBB 880-9836

Fig. 1A



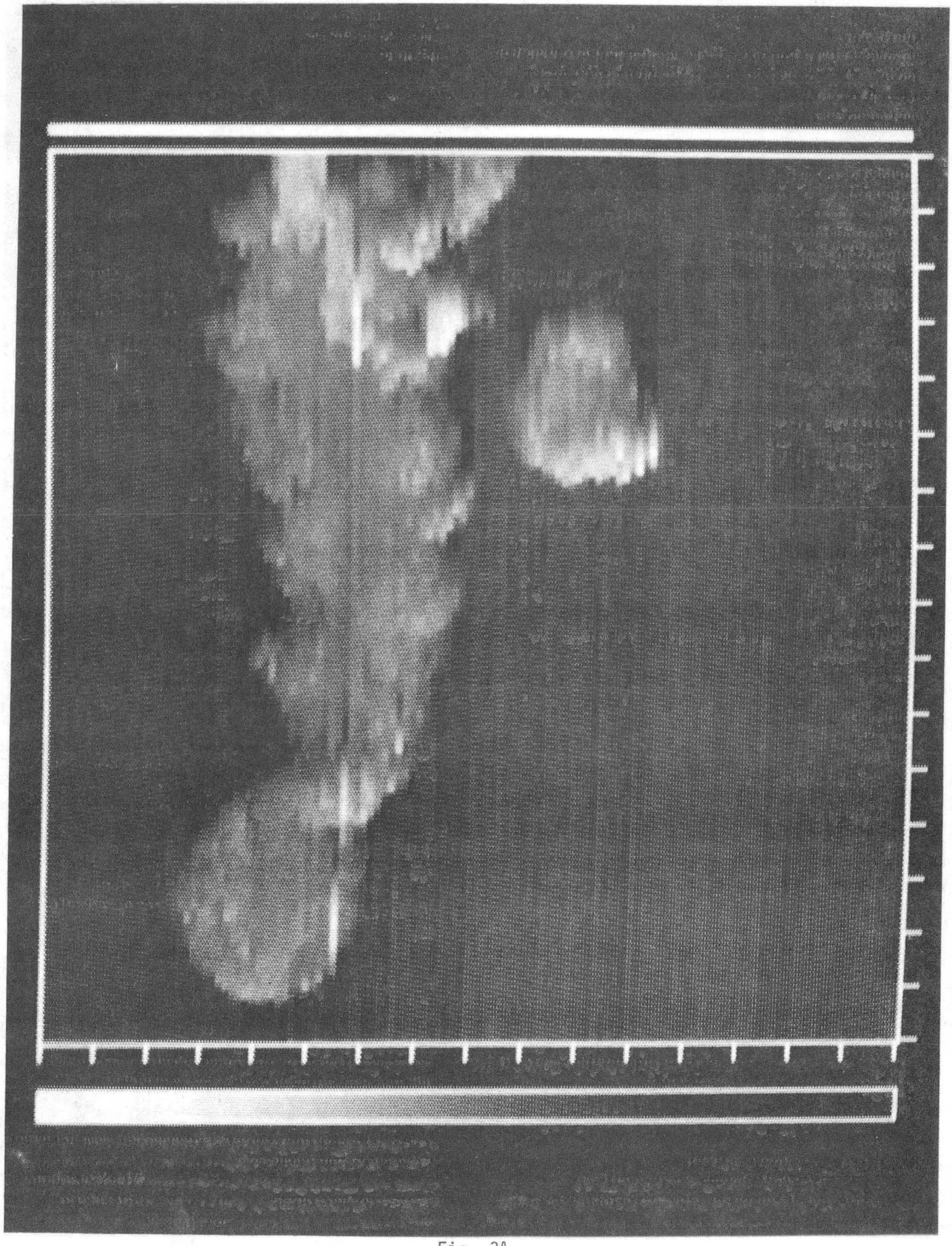
XBB 880-9837

Fig. 1B



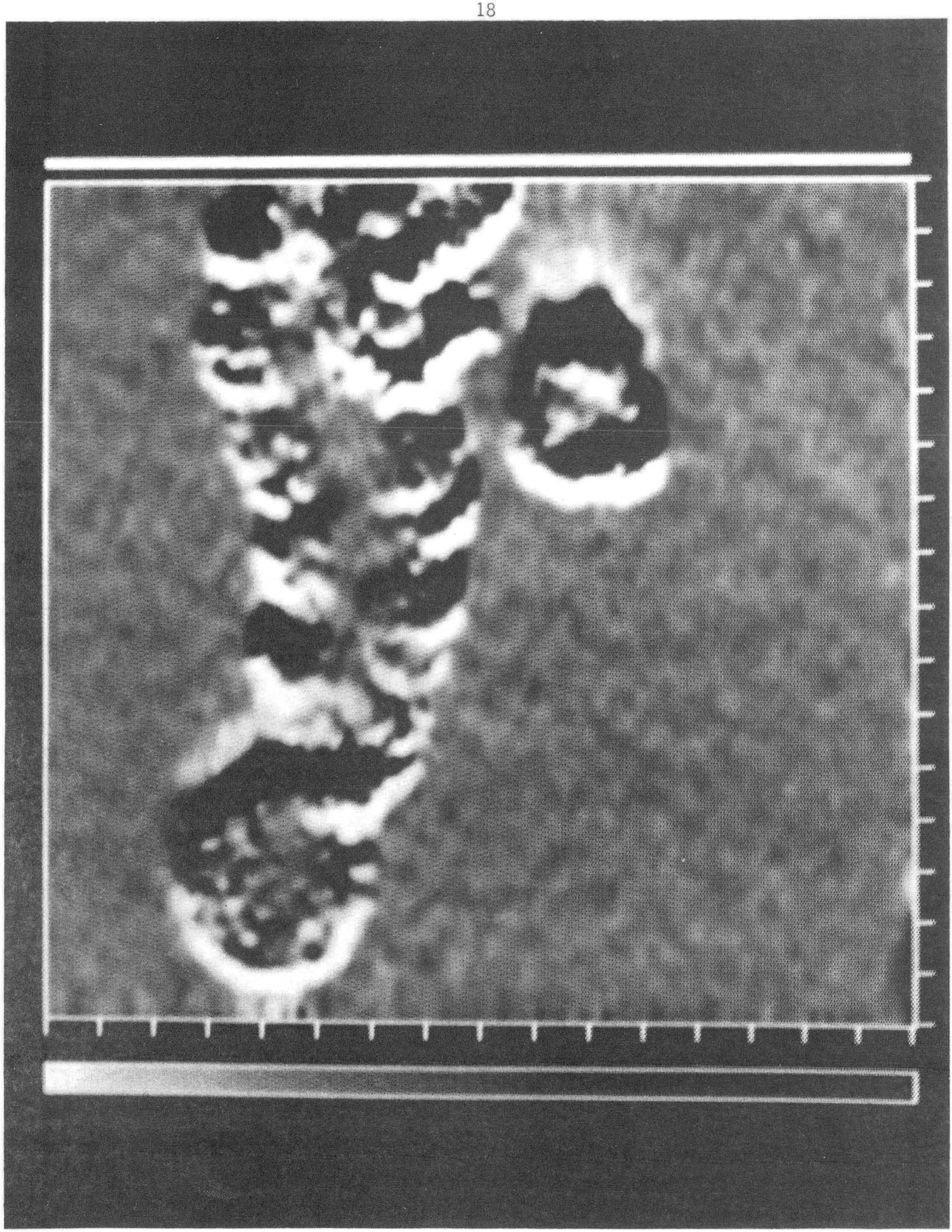
XBC 880-9842A

Fig. 2



XBB 880-9839

Fig. 3A



XBB 880-9840

Fig. 3B

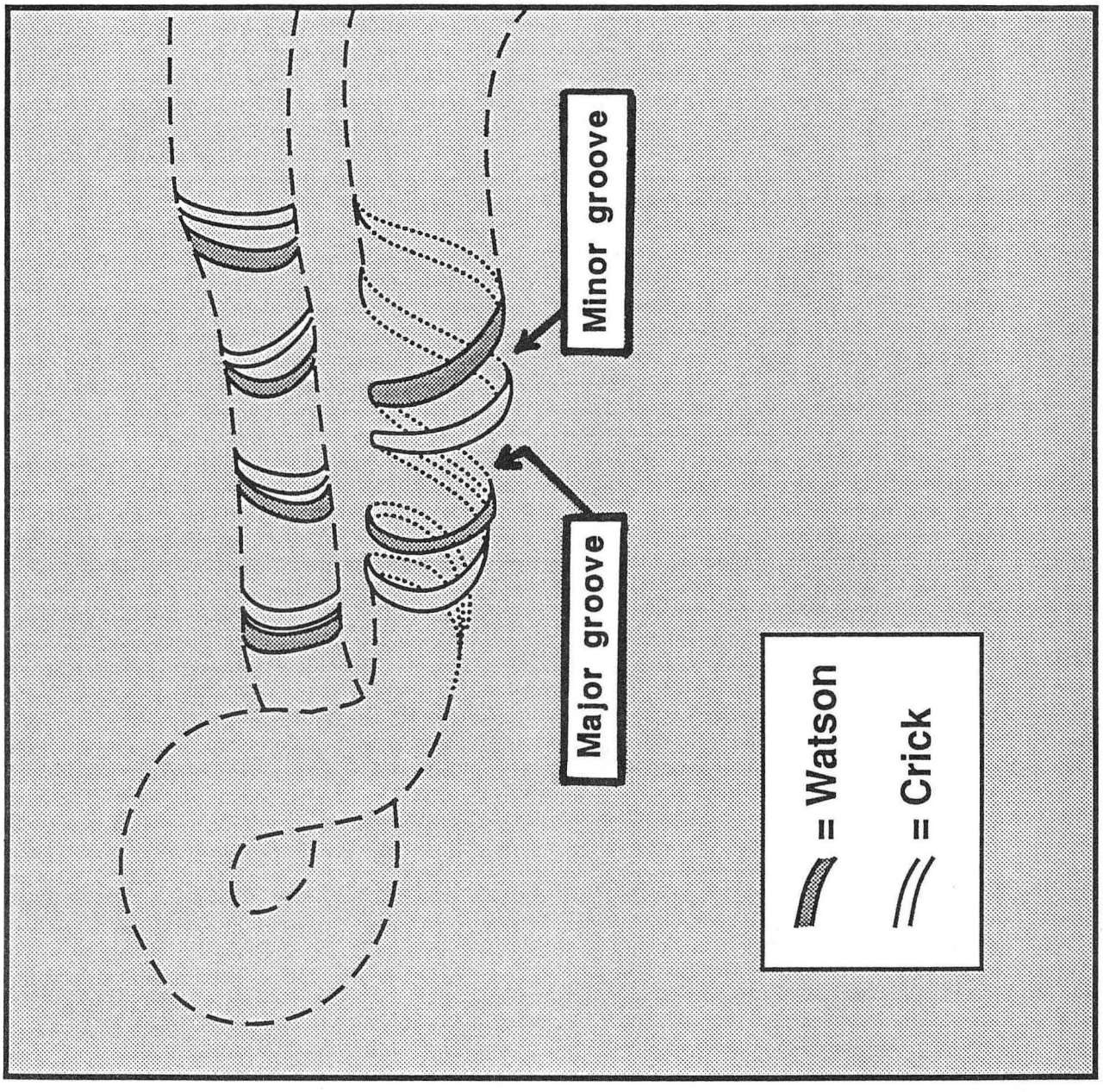


Fig. 3C

LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
1 CYCLOTRON ROAD
BERKELEY, CALIFORNIA 94720