LBL-31121

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

Engineering Division

Presented at the International Conference on Scanning Tunneling Microscopy, Interlaken, Switzerland, August 12–16, 1991, and to be published in the Proceedings

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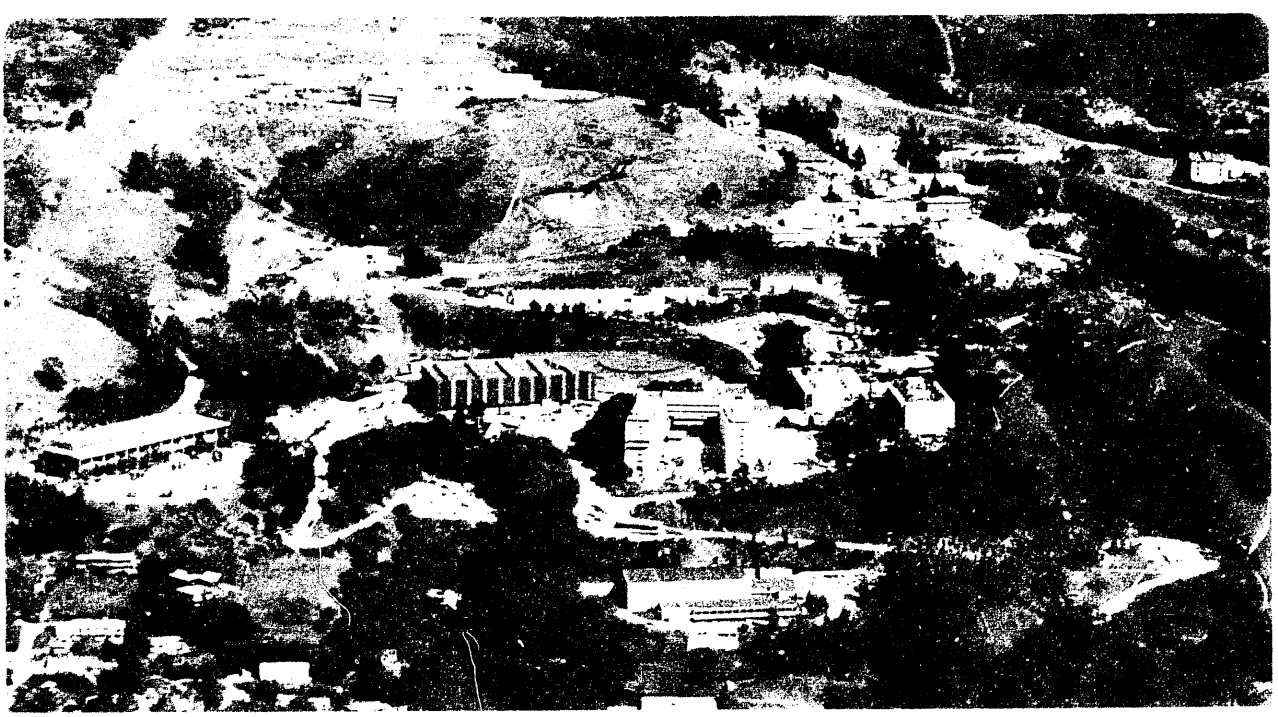
August 1991



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

LBL--31121

DE92 004159

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This work was supported by the Director, Office of Energy Research,
Office of Health and Environmental Research and Office of Basic Energy Sciences,
U.S. Department of Energy under contract DE-AC03-76SF00098.

ATOMIC FORCE MICROSCOPY IMAGING OF T4 BACTERIOPHAGES ON SILICON SUBSTRATES

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ABSTRACT

A new atomic force microscope incorporating microfabricated cantilevers and employing laser beam deflection for force detection has been constructed and is being applied to studies of biological material. In this study, T4 bacteriophage virus particles were deposited from solution onto electronic grade flat silicon wafers and imaged in air with the microscope. Microliter droplets of the solution were deposited and either allowed to dry or removed with blotting paper. The images show both isolated viruses and aggregates of various sizes. The external structure as well as strands believed to be DNA streaming out of the virus could be observed. The construction of the microscope and its performance are also described.

INTRODUCTION

Both the scanning tunneling and atomic force microscopes (STM and AFM) have been used to image a wide range of biological structures. In the case of the STM, the requirement of electrical conductivity has limited observations to metallized specimens or to very thin samples. The ability of the AFM to image non-conductive objects in air or in buffer solution has made it an attractive instrument for biological imaging [1-4].

The T4 virus is a well known bacteriophage which infects the *E. coli* bacteria. According to TEM observations [5] of the T4 using metal shadowing techniques, the virus consists of an icosahedral shaped head approximately 1000 Å in diameter filled with DNA and a tail sheath of similar length but smaller diameter. Attached to the end of the tail section are a number of tail fibers a few Å in diameter and about 1500 Å in length. Because of its readily identifiable shape and size, the T4 virus is a good candidate for AFM observation. In previous studies, STM images of metal coated T7 bacteriophages (similar to the T4) have been obtained [6] and the metallized virus particles have been used [7] as a size standard for STM calibration purposes. The virus, to our knowledge, has not previously been observed with the AFM in its natural, uncoated form.

In the paper we describe a new AFM which we have constructed and illustrate its capabilities and performance. As an application of its use in the imaging of biological material, we present images of uncoated T4 virus particles deposited on flat silicon wafers. These images show both the shape of the virus as seen by the AFM and the presence of DNA strands released from the virus.

EXPERIMENTAL

Instrumentation

All measurements were performed using an AFM constructed by us and shown in Fig. 1. The microscope, similar to other recently published designs [8,9], employs a microfabricated cantilever [10] with an integrated tip and an optical lever to detect the cantilever motion. The sample is mounted on the end of a piezoelectric tube scanner with 4 segmented electrodes which provides a raster scan in the x and y directions together with motion in the surface normal (z) direction.

Light from a laser diode [11] is focused on the end of the cantilever and reflected into a 4-segment photodiode [12]. The cantilever motion is sensed by combining (via addition and subtraction) the optical signals from the 4 segments in pairs in such a way as to detect the optical beam deflection in two orthogonal directions. One direction corresponds to motion of the cantilever normal to the sample surface and the other to a twisting of the cantilever due to frictional forces encountered during the scan. The latter can be recorded along with the normal image as a measure of these frictional forces [13].

The microscope can be operated in either the constant height or constant force mode. In the former, the cantilever deflection is recorded with the piezo maintained at a fixed *z* during the scan, whereas in the latter, the cantilever deflection and hence the force is maintained constant by feeding the deflection signal back to the *z*-direction piezo supply. The constant force mode was used in all of the images in this report. The electronics and computer control software is similar to that employed in STM instrumentation we have developed and is described elsewhere [14].

Sample preparation

The virus samples were deposited on substrates made from polished silicon wafers of semiconductor grade. Such wafers have been found [15] to be flat to within a few Å over areas of several thousand Å. In addition, the SiO_2 surface of the wafers is similar to glass to which proteins and other biological samples are known to adhere. Prior to use, the wafers were cleaved to an appropriate size and cleaned with an H_2SO_4/H_20_2 etch (piranha) to remove organic contaminants.

T4 virus particles were obtained [16] suspended in a saline solution at an initial concentration estimated to be about 10^{14} viruses/ml. A number of techniques were investigated to deposit the virus onto the Si substrates. Direct deposit of the saline virus solution was found to be unsatisfactory because of the excessive salt residue left behind. Better results were obtained by dialyzing the saline virus solution against distilled water to remove most of the salt. The resulting nearly salt-free solution was then diluted to an estimated 10^{12}/ml before application. Droplets of virus solution (0.5 - 1.0 μl) placed on the Si and allowed to dry tended to aggregate into small spots. However, in the regions near these spots, appropriate regions of suitable concentration for imaging could be found.

Virus samples were also prepared [17] by the following method: a 0.5 to 1.0 μl deposit of the dialyzed virus solution was deposited on the Si surface, forming a droplet about 1 mm in diameter. The virus particles were allowed to settle for about half a minute, after which the droplet was dried by wicking it from one edge with Whatman filter paper. The effect of the wicking is to cause the water to rush towards the filter paper, leaving a fraction of the viruses behind. This sudden onrush of liquid is believed [17] to shock the viruses, tipping them over and causing a fraction of them to spill out their DNA in the streaming direction.

RESULTS AND DISCUSSION

Performance of AFM

The noise sources affecting the optical beam deflection AFM have been discussed by Meyer and Amer [13], who have derived an expression for the contribution due to shot noise in the diode detector. For our laser with its beam waist diameter of about 10 μm,

cantilever length of 100 μm and reflected laser power of approximately 50 μW into each half of the diode detector, their calculation yields a shot noise contribution corresponding to $\Delta z=10^{-3}$ Å $/\sqrt{Hz}$. With the cantilever in contact with a silicon test surface and the feedback loop in operation, we measured the noise spectrum using an FFT power spectrum analysis built into our microscope control software. The results, shown in Fig. 2, are in reasonable agreement with the estimated noise contribution from the shot effect for frequencies above 1000 Hz. At lower frequencies the noise increases approximately as 1/f, due to environmental noise and pointing instabilities of the laser beam. The total averaged noise over our operating bandwidth of 5 kHz is 0.75 Å. The observed noise level during an actual scan is slightly larger.

Figure 3 shows an atomic resolution image of a cleaved mica surface taken in air with a with a cantilever force of ~$5x10^{-8}$ N. Rather large corrugations of 5 Å peak-to-peak were observed. This enhancement is likely a result [18] of the elasticity of the mica structure or an artifact arising from a twisting of the cantilever due to frictional forces. The average spacing of the holes between atoms is about 5.5 Å although the distortion of the hexagonal lattice seen is somewhat larger than previously observed [4] for mica with the AFM. Although our AFM was calibrated using a replica grating with a period of 2780 Å, calibration errors for this small scan area could be present.

T4 bacteriophage observations

Examples of T4 virus images seen in air are shown in Fig. 4. The samples were prepared as described above by depositing 0.5 μl of dialyzed T4 solution at a concentration of 10^{12} /ml on the silicon substrate and, after waiting 30 sec., removing the liquid by wicking from one edge. In Fig. 4a, which shows a single uncoated specimen prepared in this manner, the head and tail structures of the virus can plainly be seen.

Another example in which the head and tail of the virus are clearly in evidence is shown in Fig. 4b. Because of the pyramidal shape of the cantilever tips and the height of the virus, the images obtained are actually a convolution of the two shapes. As a result of this tip shape effect, the lateral dimensions of the virus images are somewhat larger than those seen in TEM studies.

Figures 4c and d show images of the viruses in which long strands approximately 35 Å in height and 160 Å in width can be seen. (In order to provide sufficient contrast to reveal these relatively small structures, a simulated light source has been provided, producing a shadow at 10 degrees to the horizon.) The strands are believed to be bundles of DNA which have streamed out of viruses which have lysed due to osmotic shock or surface tension forces generated during the sample preparation process. Bundles of DNA streaming out of the virus are also seen [17] in TEM images of viruses shocked by the filter wicking technique. In most of the images in which such DNA bundles can be seen, the viruses themselves are deformed in shape and rarely more than 450 Å in height, whereas the intact viruses have heights on the order of 1000 Å, retain their characteristic shape and do not have DNA associated with them. These observations are consistent with the idea that, in the normal virus, the DNA is packed into the head giving it sufficient rigidity for AFM observation. On the other hand, in the lysed virus, the head is collapsed and non-rigid in structure. Lysed viruses are seen in TEM images as hollowed out shells and are often referred to as ghosts.

SUMMARY

A new AFM employing microfabricated cantilevers has been constructed and used to image, in air, T4 bacteriophages deposited on flat silicon substrates. Both intact viruses which retain their characteristic shape and lysed viruses which appear deformed and

flattened out are seen. In the case of the lysed viruses, bundles of DNA can be seen streaming out from the viral heads. In spite of the large transverse forces generated during imaging, the DNA strands were not swept away, probably due to the large number of strands likely present in each bundle. Individual isolated strands of DNA were not seen on the silicon. To image isolated DNA strands on silicon, attachment via deposited films with appropriately modified surfaces and operation of the microscope under appropriate liquids to reduce the interaction forces will probably be necessary as recently reported [19].

ACKNOWLEDGEMENT

The authors would like to thank M. Maestre for providing the T4 samples and for helpful advice in their manipulation. Thanks are also due to L. Sindelar for assistance in sample preparation and P. Vallotton for assisting in some of the measurements. This work was supported in part by the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the Department of Energy to the exclusion of others that may be suitable.

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FIGURE CAPTIONS

Fig. 1 Cross-sectional view of AFM head showing major components.

Fig. 2 AFM noise power spectrum. The noise voltage was measured at the output of the feedback loop, Fourier transformed to obtain the power spectrum and converted to Å $/\sqrt{Hz}$ using the z-piezo voltage calibration.

Fig. 3 Atomic resolution image of cleaved mica surface.

Fig. 4 AFM images of T4 bacteriophages deposited on silicon: in (a) and (b) 3D perspective views of intact viruses and in (c) and (d) images of lysed viruses with long strands believed to be bundles of DNA are shown.

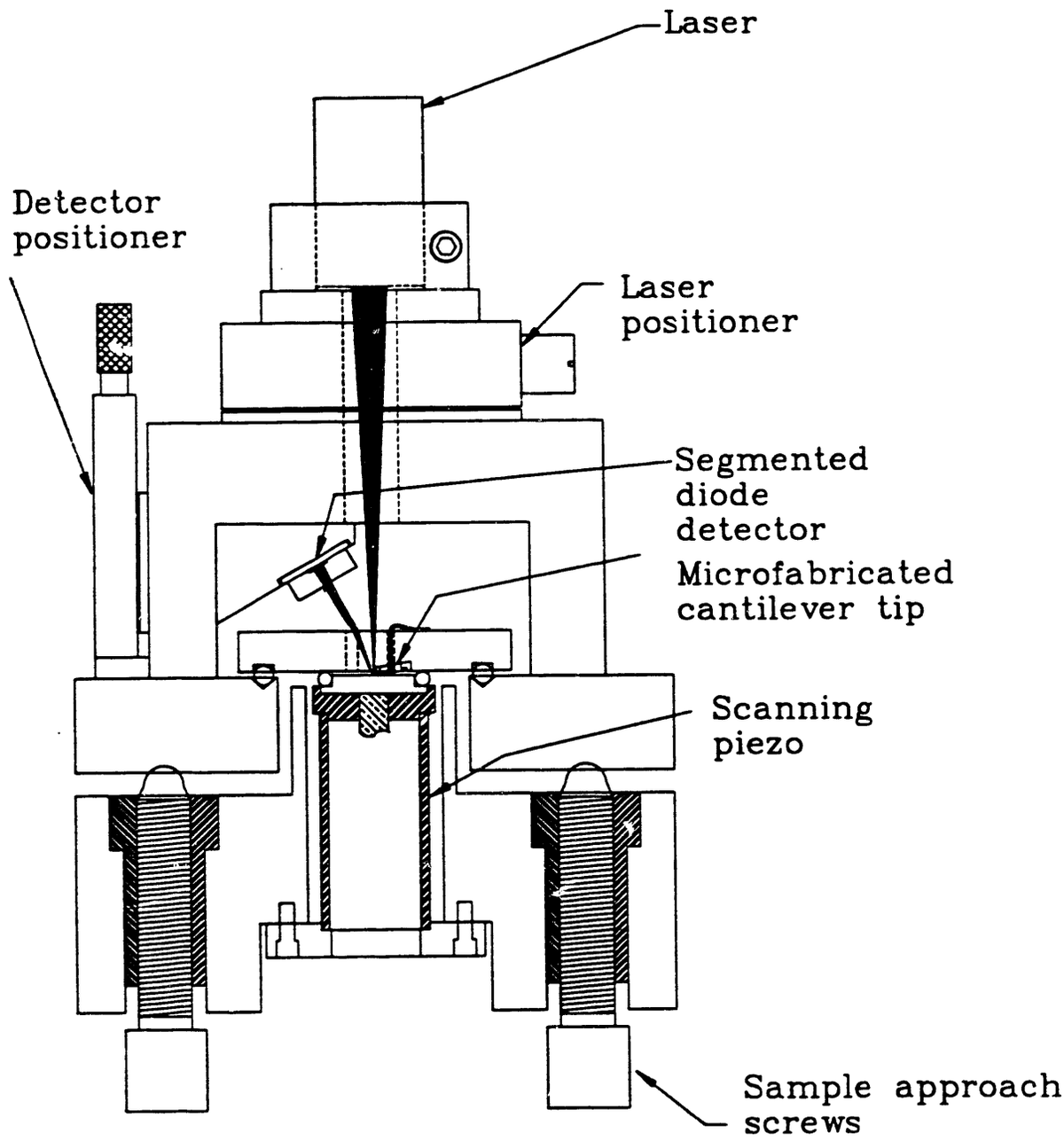

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FIGURE 1

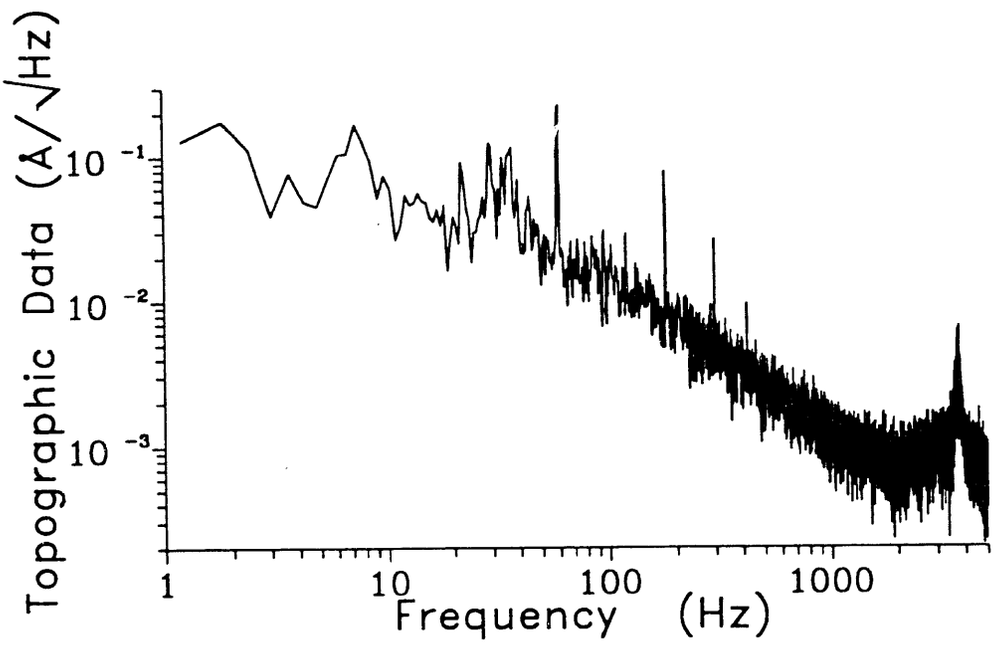

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FIGURE 2

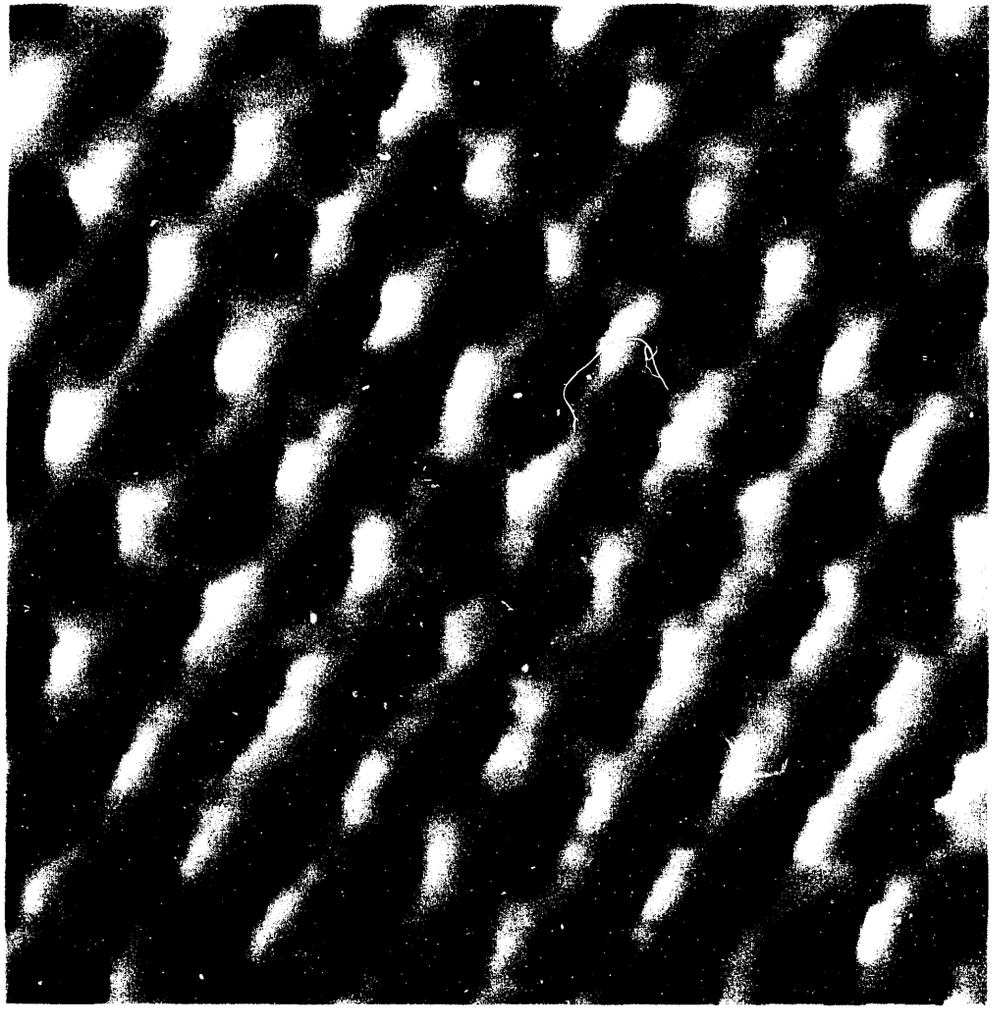

FIGURE 3

FIGURE 4A

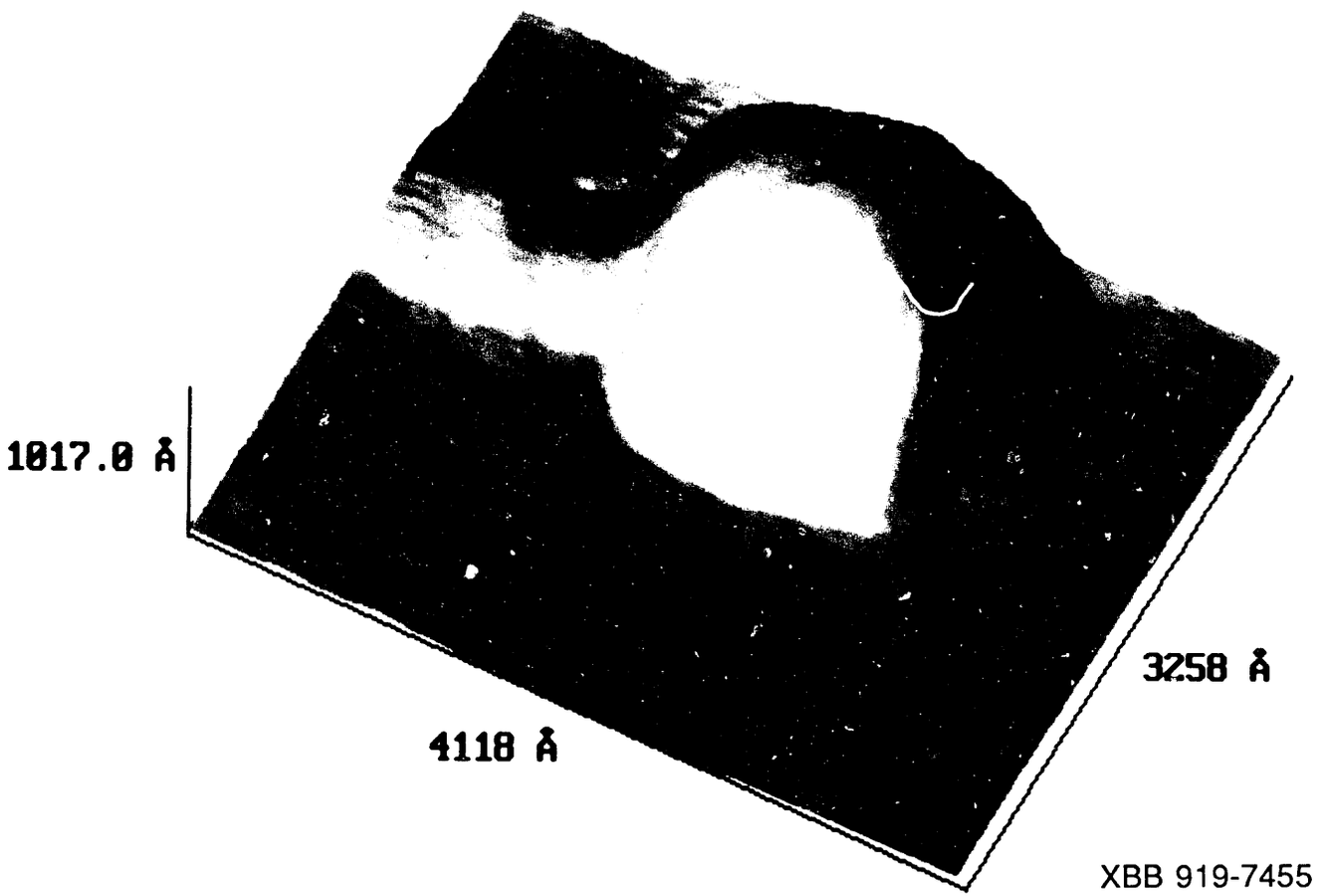

FIGURE 4B

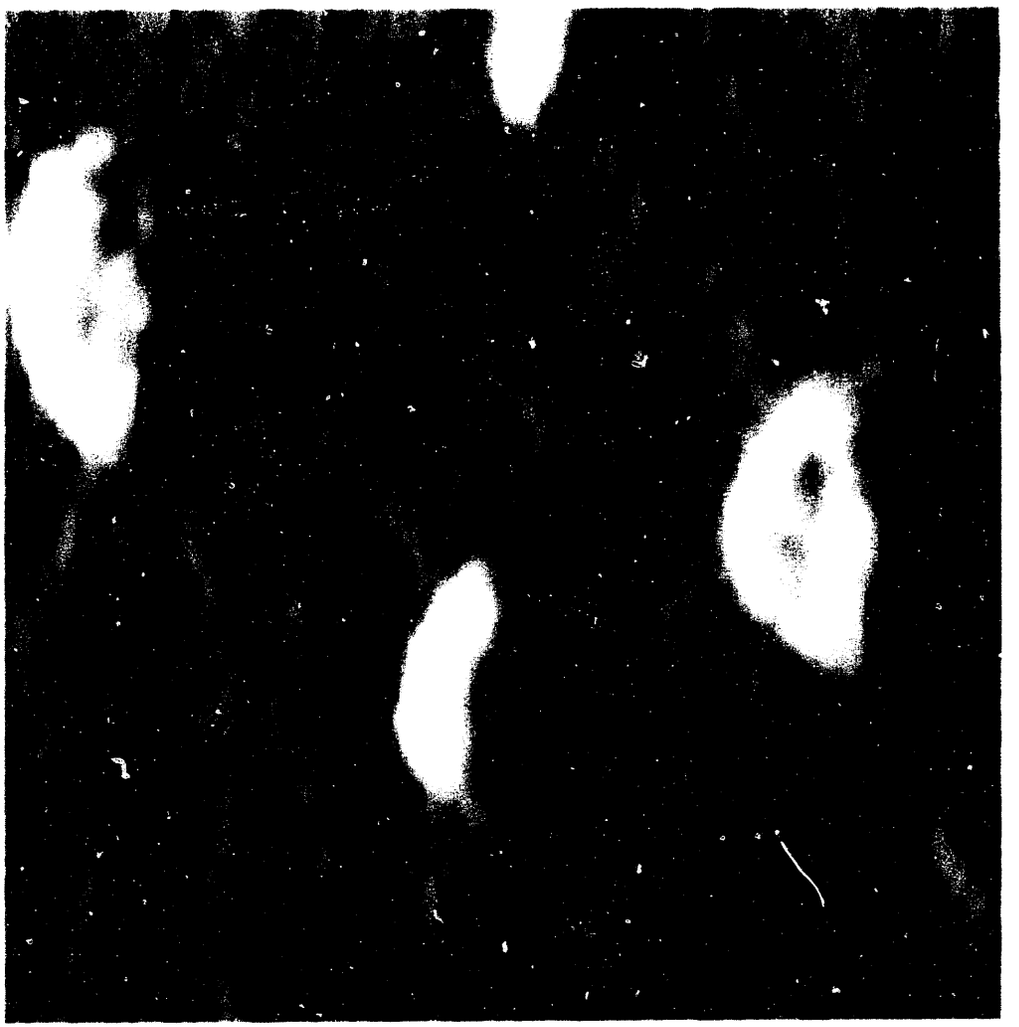

FIGURE 4C

12029 Å

12022 Å

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FIGURE 4D

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