

# Images of a lipid bilayer at molecular resolution by scanning tunneling microscopy

(biological membranes/cadmium isocanoate/graphite)

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**ABSTRACT** The molecular structure of a fatty acid bilayer has been recorded with a scanning tunneling microscope operating in air. The molecular film, a bilayer of cadmium isocanoate (arachidate), was deposited onto a graphite substrate by the Langmuir-Blodgett technique. The packing of the lipid film was found to be partially ordered. Along one axis of the triclinic unit cell the intermolecular distance varied randomly around a mean of 5.84 Å with a SD of 0.24 Å. Along the other axis the mean distance was 4.1 Å and appeared to vary monotonically over several intermolecular distances, indicating that a superstructure of longer range may exist. The molecular density was one molecule per 19.4 Å<sup>2</sup>. The surprising ability of the scanning tunneling microscope to image the individual molecular chains demonstrates that electrons from the graphite can be transferred along the molecular chains for a distance of 50 Å.

Molecular monolayers, bilayers, and multilayers are important in the fields of biology and technology. In biology where they occur as lipid membranes the details of the two-dimensional packing are important for our understanding of their biological function (1). The proposed technological uses of Langmuir-Blodgett films as thin film dielectrics make it necessary to characterize them if we are to optimize the interactions that occur within the film and at the interfaces with the environment (2). In recent years a number of techniques have been applied toward the problem of determining the structure, orientation, and packing of molecular monolayers and bilayers. The methods that have been used to characterize these films include infrared spectroscopy (3), Raman spectroscopy (4), electron diffraction (5), x-ray diffraction (6), and fluorescence microscopy (7). However, even for the simple cases of pure fatty acids, it is difficult to unambiguously determine the packing structure. The most direct way to study molecular packing is with a microscope that is capable of resolving the intermolecular distances, which in the case of the fatty acids is about 5 Å. In this report we will demonstrate that the scanning tunneling microscope (STM) is useful for this purpose. A preliminary report on this work has been presented elsewhere (25).

The technique of scanning tunneling microscopy has been used to image the distribution of electronic charge density for a variety of conducting and semiconducting solid surfaces (8-10). Viruses (11) and DNA (G. Binnig, H. Rohrer, E. Courtens, H. Gross, and J. Sogo, personal communication) deposited on conducting substrates have also been imaged with the STM, but the resolution in these experiments was only about 50 Å. In this report we present STM studies of a fatty acid bilayer deposited on a graphite substrate; we are able to reproducibly image the individual molecules in the film with a resolution of about 2 Å. By using the STM in a

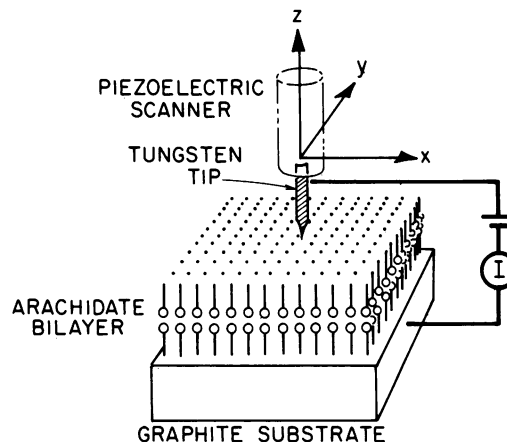


FIG. 1. Schematic of the STM experiment. A fine tungsten tip is brought to within several angstroms of the sample surface. With a voltage applied to the tip, electrons can tunnel across the gap, and a current is detected at the sample. If the tip is now rapidly scanned over the sample in the x and y directions, the variations in the tunnel current correspond to the atomic corrugation of the surface.

high-speed mode we have obtained several thousand pictures that can be assembled into a "movie." The lipid film, prepared by the Langmuir-Blodgett technique, consists of two monomolecular layers of cadmium isocanoate (cadmium arachidate; CdAch), each 27 Å thick. High resolution imaging of organic molecules has been possible with the STM because, to our surprise, the bilayer of closely packed fatty acid molecules had sufficiently high electrical conductivity.

The STM experiment is shown schematically in Fig. 1. A finely sharpened tungsten tip is brought to within several angstroms of the surface of our sample by means of a coarse mechanical approach and a fine piezoelectric transducer. With a voltage applied to the tip, electrons can tunnel across the gap, and a current is induced. This current decreases exponentially as the gap spacing increases and provides an extremely sensitive measure of the tip-to-sample distance. Height changes of 0.1 Å are easily detected. In our method of imaging (12) we raster scan the tip over a flat area of the sample while maintaining the tip at an average distance. The detected tunnel current then contains variations that correspond to the atomic corrugation of the surface. The current variation, representing surface contour, is converted to a video signal that maps the surface over which the tip scans. In this way we take television "movies" of the atomic surface structure at a rate of about 10 frames per sec.

Abbreviations: STM, scanning tunneling microscope; CdAch, cadmium isocanoate (cadmium arachidate).

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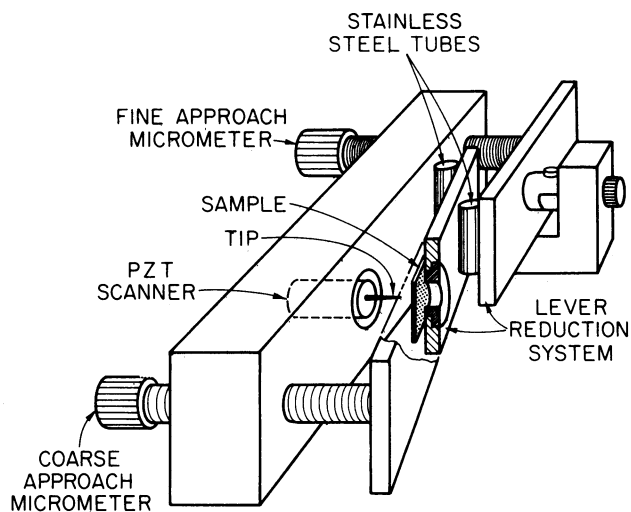


FIG. 2. The STM used in this study. The sample is moved toward the tungsten tip using a pair of micrometers and a lever reduction system. The fine-distance control and the scanning are performed by a single piezoelectric tube with four electrodes on the outside and one on the inside.

The microscope itself is quite simple (Fig. 2). The coarse-approach mechanism is a pair of micrometers whose motion is reduced by a system of levers. The sample can be brought toward the tip from several millimeters away with a resolution of about 200 Å. The fine-distance control and the scanning is performed by a single piezoelectric tube with four equally spaced electrodes on the outside and one electrode on the inside. Motion in three orthogonal directions is possible by applying voltage to the proper electrodes (13). To filter out ambient vibrations, the microscope is mounted on top of stacked metal plates separated by pieces of rubber. The microscope can operate in air at room temperature.

Graphite was chosen as the substrate in this work because previous STM studies have shown that cleaving gives large regions that are atomically flat (11, 12, 14, 15). Moreover, in air the surface appears to remain clean over long periods of time. The fatty acid bilayers were fabricated using the Langmuir-Blodgett technique (16), shown schematically in Fig. 3. Icosanoic (arachidic) acid dissolved in chloroform was spread on the surface of water and compressed to a surface pressure of  $30 \text{ mN}\cdot\text{m}^{-1}$  in order to create a monolayer. The water contained  $2.5 \times 10^{-4} \text{ mol/liter}$  of  $\text{CdCl}_2$  at a pH of 7 to stabilize the monolayer as the cadmium salt. Freshly cleaved samples of highly oriented pyrolytic graphite (provided by A. W. Moore, Union Carbide, Parma, OH) were lowered into the water at a speed of about 3 mm/min. Because graphite is hydrophobic, one monolayer was deposited on the way into the water, and a second one was added on the way out. The transfer of the monolayer on the downstroke was somewhat smaller than unity and was close to unity on the way up. The presence of the film was checked by ellipsometry; the average thickness was found to be close to a double layer. Attempts to transfer a single layer were unsuccessful because graphite is too hydrophobic to allow a transfer with the hydrophilic head group on the graphite. The configuration with the head groups facing the air is also unstable. Recently it has been shown that the STM can operate underwater (17). This should make imaging of a monolayer possible by having the hydrophobic tail on the graphite and the hydrophilic head in the water where it would be stable.

Fig. 4 shows a comparison of an image of cleaved pristine graphite and an image of a CdAch bilayer deposited onto cleaved graphite. Images such as these have been repeated several times with different samples. In both pictures in Fig. 4 the tip voltage was 40 mV, positive with respect to the sample, and the average current was held to 2.0 nA. The picture was

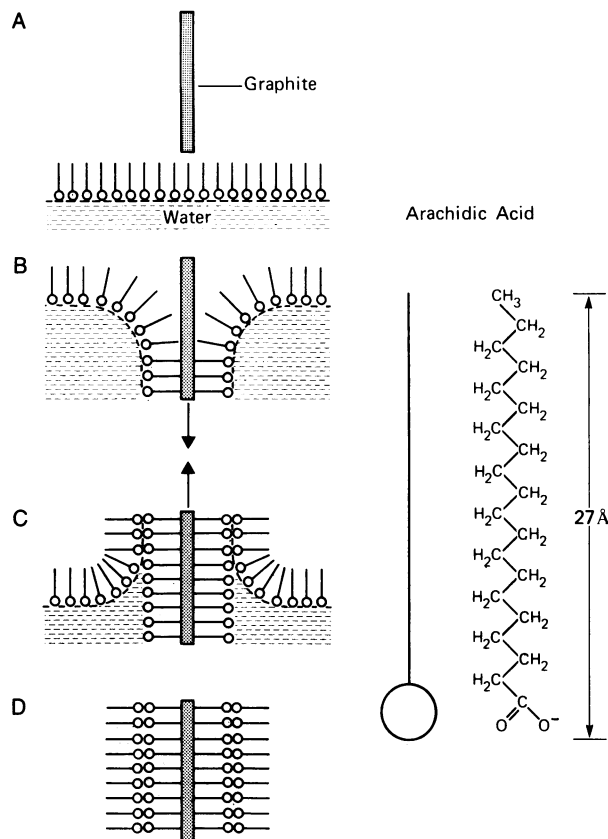


FIG. 3. Sample fabrication with the Langmuir-Blodgett technique. Arachidic acid dissolved in chloroform is spread on a water surface (A). The graphite substrate is lowered into the water, and because it is hydrophobic, it gains a monolayer on the way down (B). A second monolayer is transferred on the way up (C), giving a CdAch bilayer on graphite in air (D).

scanned at 2 kHz in the horizontal direction and 80 Hz in the vertical direction. The image was converted to a video signal and stored on video tape using an Arlunya image-processing system (Princeton Electronics Products, North Brunswick, NJ). It was then temporally filtered using the same machine and spatially filtered by a digital computer. The distortion introduced into the picture due to scanner nonorthogonality and unequal scan ranges was removed by linearly transforming the graphite image to agree with its known hexagonal structure (18); the transformation required to correct for scanner imperfections is constant with time. The same transformation was applied to the image of the Langmuir-Blodgett film. Because the experimental parameters in the two images are the same (the same tip was used), the corrected graphite image serves as a calibration for the Langmuir-Blodgett picture.

The CdAch film in the eyes of the STM looks qualitatively different from the graphite substrate onto which it is deposited. The graphite has a very uniform structure, while the Langmuir-Blodgett film shows irregularities in both the apparent size of the molecules and the distances between them. To show the larger-scale structure of the molecular packing we have assembled a sequence of four images of the Langmuir-Blodgett film (Fig. 5). These four images were taken from a video recording that showed the gradual movement of the Langmuir-Blodgett film through the viewing field of the microscope. Whereas the graphite shows a variation in lattice spacing of only about 1%, the Langmuir-Blodgett film shows a significantly greater variation and more anisotropy. The observed packing fits a triclinic unit cell. The intermolecular distances along one axis, the *a* axis, are larger than along the other axis, the *b* axis.

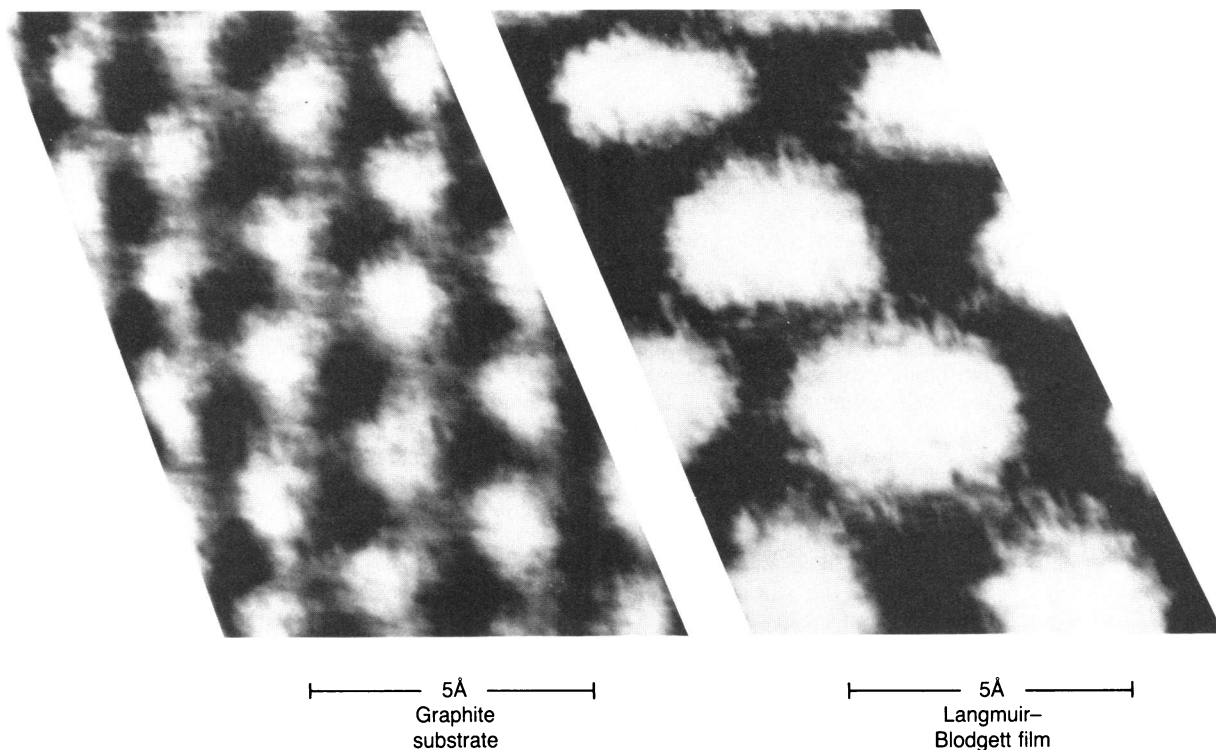


FIG. 4. Images of a cleaved graphite sample obtained with a STM. *Left*, pristine graphite. *Right*, graphite coated with CdACh bilayer. Brightness indicates higher tunneling current and therefore represents higher topography, or increased conductivity.

The two axes form a mean angle of  $128^\circ$  with a SD of  $2^\circ$ . The variation along the  $a$  axis is random, but the distances along the  $b$  axis vary monotonically. Using the graphite C-C bond length of  $1.42 \text{ \AA}$  from Fig. 4 as a distance calibration, we find the average intermolecular distance along the  $a$  axis to be  $5.84 \text{ \AA}$  with a SD of  $0.24 \text{ \AA}$ , or 4%. The variation of the lattice spacing is consistent with the electron diffraction linewidth

for lipid monolayers on silicon oxide surfaces, where coherence lengths of 50 to  $100 \text{ \AA}$  were observed (6). This coherence length would predict a 5% variation in the intermolecular distances, and this is consistent with our findings. The spacings along the  $b$  axis are smaller, with a mean of  $4.1 \text{ \AA}$  and a SD of  $0.43 \text{ \AA}$ . However, this average is not meaningful since the  $b$  axis spacings decrease monotonically from the bottom

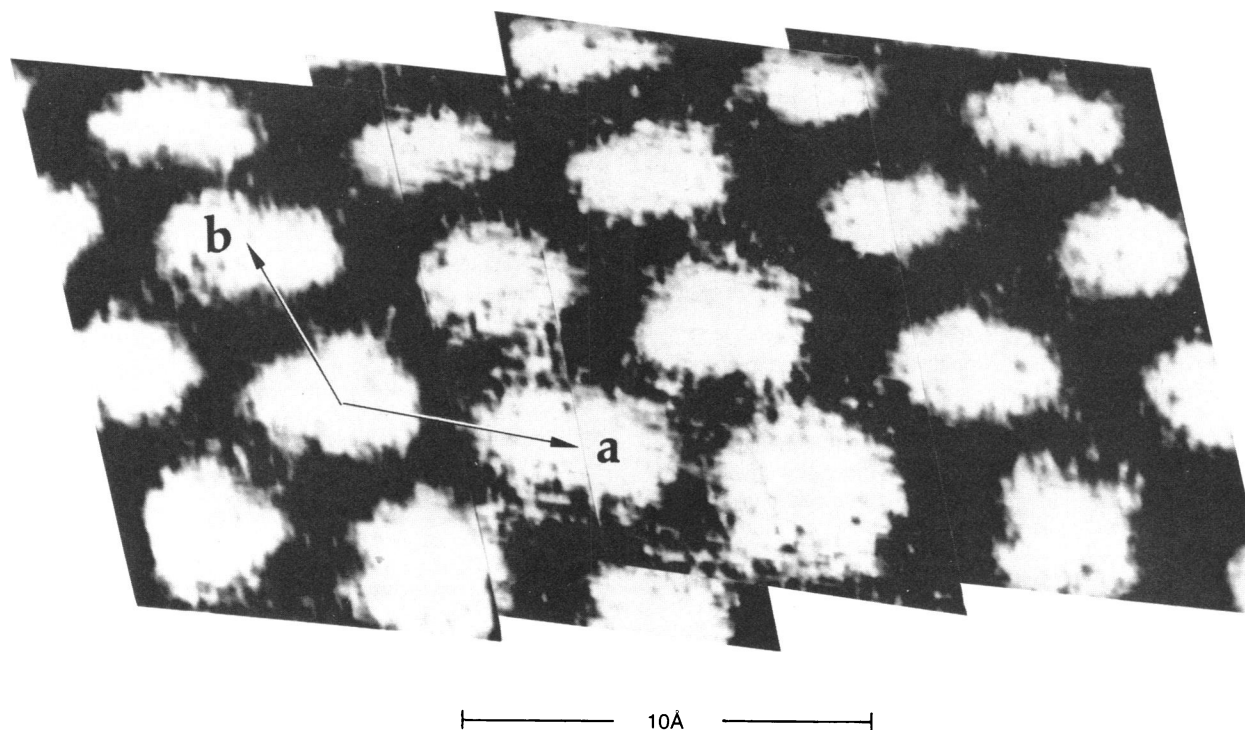


FIG. 5. STM image of a CdACh bilayer. The variation along one axis ( $a$ ) of the triclinic unit cell is random with an average of  $5.84 \text{ \AA}$  and a SD of  $0.24 \text{ \AA}$ . The spacings along the other axis ( $b$ ) are smaller, about  $4 \text{ \AA}$ , and monotonically decrease from the bottom to the top of the image. The mean area of the unit cell is  $19.4 \text{ \AA}^2$ .

to the top of the image. Larger areas need to be investigated in order to find whether a possible superstructure exists (19). The mean molecular density is one molecule per  $19.4 \text{ \AA}^2$ . This agrees very well with the calculated density of molecules on the water surface from pressure-area curves (ref. 16, p. 186).

The ordered Langmuir-Blodgett film was consistently observed to flow across the viewing field of the STM. In the sequence shown in Fig. 5 the CdACh lattice appeared to move at a rate of about  $4 \text{ \AA}/\text{sec}$ . Because images of pristine graphite were free from drift over several minutes, the Langmuir-Blodgett film might be actually sliding across the graphite substrate. Some areas of the samples coated with the Langmuir-Blodgett molecules showed no ordered packing of the molecules: in these areas large unidentifiable lumps of material were seen sitting on top of the graphite substrate. The lumps were observed to move relative to the stationary graphite lattice and may be disassociated CdACh molecules. In some places only the graphite substrate was visible on the coated sample. In contrast to images of pristine graphite that were very stable, the clarity and resolution of the Langmuir-Blodgett images would change suddenly. These effects indicate that the film is nonuniform over the whole sample, possibly because of weak bonding to the substrate. No edge separating the ordered molecular film from the graphite substrate could be located.

The morphology of these lipid films, which was the focus of this study, is not the only interesting feature of the Langmuir-Blodgett films. We found that current at the position of the tip could be easily measured, and this suggests that the STM technique is useful for studying charge transfer in organic solids. Hopfield (20) has described the transfer of charge along molecular chains where the electronic states are localized at the position of the atomic sites. The electronic properties of these molecular assemblies are very different from those in crystalline solids. The high degree of periodicity that gives long-range order in crystalline solids is nonexistent in molecular solids, and as a result, the electrons are believed to be confined to the molecular chains. This condition is evident in our images, because they show little conduction in the regions between the molecules. Another effect to consider is the weak van der Waals forces between chains that can lead to fluctuations in the molecular conformations along the chains. We found that the current was significantly noisier when imaging the Langmuir-Blodgett films than it was with pristine graphite; this may be a manifestation of molecular fluctuations within the film.

With the STM we were able to examine well-defined areas of the sample and control the gap spacing precisely. Let us consider that the total current was conducted along a CdACh filament with a cross-section of  $20 \text{ \AA}^2$ , the approximate resolution we observed. Then for a current of 2 nA and voltage of 40 mV the resistivity of the  $50 \text{ \AA}$  chains was  $10^3 \Omega\text{-cm}$ . In contrast to this fairly low resistivity, other experiments (21, 22) have suggested that the conduction through fatty acid monolayers on oxidized metal substrates occurs by tunneling through an insulator with a resistivity ranging from  $10^9$  to  $10^{15} \Omega\text{-cm}$ . While these results using planar tunneling junctions show that the electron affinity of the films acts to significantly lower the tunneling barrier, it would appear unlikely that tunneling alone could account for the high conductivity we observed. Baró *et al.* (11) in their STM experiments with viruses have also found surprisingly high conductivity through thick organic materials. They propose the existence of conduction levels lower than the kinetic energy of the electrons, so that the electrons can propagate, rather than tunnel, through the specimen. Duke has dealt with this question at length in his theoretical treatment of conduction through organic materials (23) and in his discussion of field emission from clean surfaces covered with adsorbed molecules (24).

Our results, which display the structure of the lipid bilayers in a way that is not possible with other techniques, demonstrate the advantages of using the STM to study organic molecules. In particular, we are able to obtain a resolution of several angstroms using a simple instrument. In conventional electron microscopy it is often necessary to "decorate" the molecules with metal atoms to render them visible to the microscope. With the STM this procedure is unnecessary. The high energy of the electrons, usually keV, in electron microscopes often damages the specimen. In contrast, the low energy of tunneling electrons, on the order of 10 meV, makes it possible to probe the structure without disrupting the sample. Another attractive feature of the STM is its ability to take real time movies and observe dynamical behavior. Finally, the images are taken in air rather than the vacuum environment demanded by most high-resolution techniques. Our results, combined with the recent report of the operation of the STM in water (17), suggest that it is possible to image organic molecules in an aqueous solution. This is the natural environment for many organic molecules, and it should open up a variety of interesting examinations of structural and electrical properties. Biological membranes, close relatives of the Langmuir-Blodgett films, should be particularly appropriate subjects for study.

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