Precision height measurements of freeze fracture replicas using the scanning tunneling microscope

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We propose a general model for the interaction between a scanning tunneling microscope tip and a surface being imaged in air. The van der Waals force and a liquid bridge formed from condensation cause a net attraction between the tip and sample. If the sample is flexible or weakly bound to the scanning tunneling microscopy (STM) base our model predicts this attraction can lead to a significant amplification of the height of surface features. STM images of a freeze-fracture replica of a mica surface clearly show the amplification. Freshly made replicas of an atomically smooth mica surface had a standard deviation in their heights of 0.51 nm, but this increased to 0.61 nm after five days in a Petri dish and to 1.64 nm after one day of exposure to air. Methods of further reducing the amplification are discussed.

I. INTRODUCTION

Freeze-fracture replication was first developed in 1950 and has since become a standard technique for preparing soft, fluid materials such as tissue and membranes for visualization with transmission electron microscopy. \(^1\) In this process, a thin layer of metal is deposited on to the surface of a rapidly frozen, then fractured, sample under high vacuum. The metal replica of the surface can then be removed, cleaned and imaged, typically with a transmission electron microscope. Recently this technique has been used to image non conducting biological surfaces with scanning tunneling microscopes (STM). \(^3\) \(^4\) which was developed by Binnig et al. in 1982. \(^5\) While lateral measurements have been easily reproduced and are in good agreement with and of superior resolution to those obtained using electron microscopy, vertical measurements have yet to approach the resolution and reproducibility that the STM is capable of providing when directly imaging hard crystalline samples. In our previous work, we have observed that height measurements of a presumably homogeneous surface could vary with location on a replica and could also change significantly when imaging the same area over the course of several days.

For the replica/STM technique to become a useful tool in studying biological structures the reliability and reproducibility of the technique must be greatly improved. We are ultimately interested in studying the \(P_B\), or ripple phase of saturated phospholipids. Estimates of the ripple amplitude range from 0.8 nm to 8 nm. \(^3\) Without a more precise measurement of the relationship of ripple height to wavelength it will not be possible to accurately characterize the forces or mechanisms that lead to the ripple structure. To make an accurate measurement of the surface we must first make a replica that precisely mirrors the surface and then image that replica accurately. The first step has already been the topic of considerable study. \(^3\) \(^4\) By using a simple model of the tip-replica interaction we have determined the necessary modifications in making replicas for imaging with the STM.

The STM measures sample heights relative to some arbitrary zero, generally relative to the STM base. In an ideal STM image it is assumed that the sample is fixed to the STM base and that the sample is both incompressible and infinitely rigid. It is also generally assumed that the STM tip does not contact the specimen surface during imaging and is not mechanically connected in any way to the sample. These assumptions are not completely true for imaging in air as shown by Mamin et al., and are especially suspect for thin metal replicas or fragile biological macromolecules in air. \(^7\)

Any time a sample is imaged in air in a normal lab environment a surface layer of condensed water vapor or other liquids left over from previous treatments coat both the sample and the STM tip. When the tip is brought close enough to the specimen, a liquid bridge can be formed that mechanically connects the tip and sample as shown in Fig. 1. The surface tension of the liquid tries to minimize the overall area of the liquid bridge, so a force is generated that attempts to pull the sample and tip into closer contact. This has been observed during atomic force microscopy (AFM) imaging in air, as the liquid meniscus appears to ‘suck’ the tip towards the sample. \(^8\) If the sample is not sufficiently incompressible or rigid, the tip-sample force may be sufficient to move the surface of the sample relative to the STM base during imaging. Such motion would alter the apparent feature heights of the surface, especially for fragile surfaces such as thin metal replicas or biomolecules that are only weakly held to the STM base or to a substrate.

Coombs and Pethica \(^9\) and Mamin et al. have proposed multiple spring models for the interaction of the scanning tip and contaminant particles on the sample, to try to describe possible differences in measured \((Z^*)\) and actual \((Z)\) feature heights. We propose a modification of their model to account for the mechanical connection between tip and sample created by the liquid bridge. We hypothesize that the liquid bridge acts as a spring connecting the STM tip to the sample, which is held to the base by a second spring. In this model the scanning tip is assumed to be attached to the sample by a spring with a spring constant \(k_1\) and the sample is connected to the base by a spring with spring constant \(k_2\) as shown in Fig. 2. This combination of springs will amplify a surface feature of height \(Z\) by

\[
Z^* = Z(k_1 + k_2)/k_2,
\]

(1)
of curvature of the sample is substantially less than that of the tip, which is a good assumption for most surfaces. The third assumption is more difficult to check. Over time it seems reasonable that larger organic molecules, salts, etc. may accumulate on the surface as well as the water, changing the effective surface tension of the liquid bridge. However, as we are only interested in the order of magnitude of the interaction this will not substantially alter the result.

With these assumptions we have the following equation for the force between the tip and the sample:

\[ F = \frac{AR}{6D^2} + 4\pi R\gamma \cos \theta / (1 + D/d), \]

where \( A \) is the effective Hamaker constant of the metals in the tip and replica acting across the condensate, \( D \) is tip-surface separation, \( \gamma \) is the surface tension of the condensate, \( \theta \) is the liquid-solid contact angle, and \( d \) is the distance the tip extends into the meniscus as shown in Fig. 3. The first term represents the van der Waals attraction between the tip and the sample as mediated by water and the second is the surface tension of the liquid bridge. For the spring constant we then have

\[ k_1 = \frac{AR}{3D^3} + 4\pi R\gamma \cos \theta / d(1 + D/d)^2. \]

If we assume that the condensate is water and the tip and replica are conducting metals we have the following values: \( A = 3 \times 10^{-19} \text{ J}, \gamma = 0.073 \text{ J/m}^2 \), and \( d = (1.08 \text{ nm}) \cos \theta / \ln(P/P_r) \) where \( P/P_r \) is the relative humidity. For a tip of radius 50 nm at a distance of 1 nm from the replica in 50% relative humidity this gives 15 N/m for \( k_1 \). This is in general agreement with the range of 15–50 N/m reported by Durig et al. who estimated the spring constant between graphite

where \( Z^* \) is the measured height of the feature, that is the measured motion of the piezoelectric drive of the STM tip. For reliable imaging we need to make \( k_2 \gg k_1 \), so that \( Z^* = Z \). For most bulk solid samples \( k_2 \) is much larger than \( k_1 \) and this amplification is not significant, although images of highly oriented pyrolytic graphite in air can show an amplified corrugation height if imaging is done improperly. However, freeze-fracture replicas are very thin, and hence very flexible, and are usually only weakly supported on electron microscope grids (see Fig. 1), which are loosely fastened to the STM base. We should then expect the possibility of substantial height amplifications when imaging freeze-fracture replicas in air with the STM. Hence we need to determine how to construct replicas, support them more firmly, and know what to look for during imaging.

The forces responsible for \( k_1 \) are the van der Waals attraction between the tip and the replica and an additional force due to the liquid bridge between the tip and replica. To calculate \( k_1 \) we make the following assumptions: (i) The portion of the tip in contact with the surface can be modeled as a sphere with a radius equal to the radius of curvature of the tip; (ii) The replica surface being imaged is flat over the area of interaction; (iii) The condensate on the surfaces is primarily water. The first assumption is good so long as the radius of the tip, \( R \), is large compared to the distance, \( D \), between the tip and the sample, which is well justified for typical STM tips (\( R = 50–100 \text{ nm} \)) and surface spacings (1 nm). The second assumption will hold so long as the radius

![Fig. 1.](image1.png)  (a) Condensation forms a liquid bridge between the STM tip and the thin metal replica and draws the two together by deforming the replica and pulling it from its mount. The actual size of the mount is larger than shown. (b) When the tip pulls away from the replica to go over a surface feature of height \( Z^* \), the liquid bridge and van der Waals force pull the replica along with the tip. The STM tip must therefore retract a distance \( Z^* \), which is greater than \( Z \), in order to maintain a constant current.

![Fig. 2.](image2.png)  In the multiple spring model \( k_1 \) is the effective spring constant between the STM tip and the sample and \( k_2 \) is the effective spring constant between the sample and the base.
and the tip in air. The same values indicate a force of $1.2 \times 10^{-7}$ N is being exerted on the sample by the STM tip.

To estimate $k_2$, we will note that we originally mounted the replicas on fine mesh gold electron microscope grids or other porous surfaces to allow water to be drawn off through the mounting. In previous work we found that mounting replicas on solid surfaces was difficult and led to a reduced survival rate for the replicas. However, we continue to explore the possibility of using nonporous mounts. Where the replica is in direct, firm contact with the mount $k_2$ is relatively large. However, much of the replica is not in direct contact with the grid or the replica is not perfectly flat. If we assume that the replica is loosely suspended over a square pore of area, $a$, when the tip is located over the center of the square, $k_2$ is given by

$$k_2 = \frac{8ET^2}{a}, \tag{4}$$

where $E$ is the Young’s modulus of the replica and $T$ is the thickness of the replica.

Freeze fracture replicas prepared for transmission electron microscopy are typically less than 25 nm thick, usually 1–2.5 nm of platinum or a primary film backed by 10–20 nm of carbon, with a Young’s modulus of about $2 \times 10^{10}$ N/m$^2$, and mounted on a grid with 30 micron spacings. Thus near the center of a grid square $k_2 = 0.003$ N/m. Combined with our earlier estimate of $k_1$, this combination of springs gives an amplification by a factor of over 1000. Since many of the quantities involved cannot be accurately measured, and vary from point to point on the sample, it is not possible to precisely quantify the amplification and to arrive at a correcting factor for a height measurement. Instead we must use these equations to try and modify the replicas and mounts to make $k_2 > k_1$ and effectively eliminate any amplification. Equation (4) suggests that making the replicas substantially thicker while at the same time providing pores of smaller area, we can increase $k_2$. While thicker replicas are not compatible with the transmission electron microscopy (TEM), they make no difference to the STM, which only images the surface layer. Smaller pores in the substrate cause no inherent problems for the STM technique, and should lead to better sample support.

II. EXPERIMENT

To test the concepts behind our model and our ability to accurately replicate a surface we looked at the surface of mica. Mica was chosen because AFM images have shown that it cleaves along surfaces that are atomically smooth over large areas. Ideally, we would get an image of the replica with features no more than a few Angstroms high. To date most of our work has concentrated on increasing the value of $k_2$. Some relatively simple modifications of the technique used to prepare samples for electron microscopes can change $k_2$ by several orders of magnitude.

The mica was cleaved in air and immediately loaded into a Balzers BAF 400K/CL freeze-fracture device (Balzers; Hudson, New Hampshire). The mica was allowed to equilibrate at $-170^\circ$C under a vacuum of $10^{-8}$ torr. We then replicated the surface with 1.5 nm of a platinum-carbon mixture applied by electron beam evaporation at a 45° angle to the mica surface and followed with a 15 nm reinforcing film of carbon applied at normal incidence. After removal from the freeze-fracture device the replicas were further reinforced with 200 nm of silver by a vacuum deposition. The replicas were removed from the mica using 49% hydrofluoric acid in a technique described by Ruben, the only difference being that it often took 24–48 hours for the mica to fall from the replicas.

To mount the replicas we used SPI silver membranes with 0.2 μm pores (SPI Supplies; West Chester, Pennsylvania). The membrane was used to push the replica underwater, then pulled out with the replica on the side facing up. The replica and membrane were placed on filter paper in a Petri dish to remove excess water. Despite the 0.2 μm pore size for filtering purposes, STM and TEM pictures show that at the surface most pores are 1–3 μm across [see Fig. 4(a)]. With these modifications Eq. (4) gives a value of 400 N/m for $k_2$.

This would lead to height amplifications of about 4%. STM images were taken using a NanoScope II (Digital Instruments; Goleta, California). All the images of the replica were taken at ambient temperature, pressure, and humidity using commercially available platinum-iridium tips (Digital Instruments). Daytime conditions in the laboratory are typically 70–85°F with 40%–50% relative humidity (typical Santa Barbara weather). All images of the replica were taken with an ‘A’ scanning head with a range of 800 nm at a bias voltage of 300 mV and setpoint current of 0.49 nA. A ‘D’ scanning head with a 12 μm range was used to image the silver membrane with a bias voltage of 150 mV and a setpoint current of 0.54 nA.

III. RESULTS AND DISCUSSION

To test the role of surface contamination in height measurements we took a freshly prepared replica that had dried
for several hours in a covered Petri dish and made a series of
six 50 nm by 50 nm images from different points on the
replica. The replica was then left covered in the Petri dish for
days and six more images were made. Finally the replica
was left uncovered and exposed to air overnight and then
imaged again. All the images were put through the same
computer filters to level them and eliminate high frequency
noise. The standard deviation of the height was found for
each image after filtering, again using the NanoScope II so-
ftware. The average of the standard deviations was 0.51 nm for
the first images, 0.61 nm for the images taken five days later,
and 1.64 nm after exposure to the air. One image from both
the second and third group was discarded due to the pres-
ence of an anomalously large feature in each image. Repre-
sentative images from each group are shown in Figs. 4(b)–
4(d).

The images clearly show an amplification of the surface
features. Even the images taken on the first day consistently
have peak to valley distances of 2–3 nm which is in the range
expected for a platinum-carbon layer of average thickness of
1.5 nm. It is possible that some of this roughness is caused by
a small amount of condensation on the mica from being
cleaned in air. It is also possible that coverage with the plat-
ium is incomplete and we occasionally image through to the
carbon backing.

Prolonged exposure to the atmosphere dramatically in-
creased the measured height of the replica surfaces. We be-
lieve that the increase in amplification from the exposure to
air is caused by the further contamination of the surface by
additional water and other molecules, probably salts and hy-
drocarbons. These may change the surface tension and Ha-
maker constants in Eq. (3) and may even form weak bonds
between the tip and replica. The size of the amplifications
measured indicates a substantial increase in $k_1$, since the pa-
rameters in $k_1$ did not change. The images also show that the
lateral dimensions of the surface features were essentially the
same in all three sets of images, eliminating the possibility
that a chemical reaction was causing the platinum crys-

tallites to grow due to interaction with the contaminants or by
simple Ostwald ripening.
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IV. CONCLUSIONS AND FUTURE WORK

We have proposed a general model for the interaction between an STM tip and a surface being imaged in air. The model predicts an amplification of surface features due to the effect of the van der Waals force and the surface tension of a liquid bridge between the STM tip and a flexible sample. This amplification was observed experimentally and found to increase with exposure of the replica to the atmosphere.

Improvements in our technique have greatly reduced but have not yet eliminated the amplification. However, our model suggests that some simple improvements may be enough to achieve our desired resolution. In the future we hope to further eliminate any amplification by (i) increasing the thickness of the replica; (ii) bonding the replica more firmly to a conductive, microporous support; and (iii) reducing the role of surface contamination by environmental controls.