Alternative Method of Imaging Surface Topologies of Nonconducting Bulk Specimens by Scanning Tunneling Microscopy

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A new method of imaging surface topologies of nonconductive bulk materials, especially biological specimens, by scanning tunneling microscopy is presented. The realizability of this technique relies mainly upon surface condensation of water molecules on the specimen and substrate, with the measured current carried by ions rather than tunneling electrons. The theoretical model shows that temperature and humidity control play a crucial role in the image formation. The experimental conditions are also discussed.

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The recently developed scanning probe microscopy (SPM) has shown its great potential in the study of surface structures of biological materials [1,2]. Despite the lack of understanding of contrast mechanisms, the scanning tunneling microscopy (STM) was successfully used to image nonconducting materials [3–8] with resolutions ranging from angstroms to nanometers. Many theories [9–12] have been proposed to explain the contrast formation. Although there is a great diversity of origins, these theories all agree that the STM image of a nonducting specimen may not be directly interpreted as the surface topology of a specimen. Since direct electron tunneling is essential to STM imaging, specimen thicknesses of nonconducting materials are limited to very thin preparations of several nanometers. Even when the tunneling current is measurable, the visibility of a specimen is still a complicated matter [9–12]. The atomic force microscopy [13] developed for directly imaging surface topology relies on the repulsive tip-surface interaction. The force required for high-resolution imaging is too strong for most biological specimens to withstand [14]. To improve the applicability of SPM to bulk biological materials at almost native conditions, one must either refine the available methods or develop different imaging principles. In this Letter, we discuss a new principle of operation for the STM, which furthers this purpose.

The idea is as follows: Assume that a bulk biological specimen is adsorbed on a conducting substrate, such as graphite. The system is placed in a chamber with controlled humidity and temperature. On the specimen and the substrate, there is normally a thin film of water (a few nm or less) at moderate humidity. The film, if continuous, can be considered as an ion conductor. If an STM tip is brought into contact with the film, cf. Fig. 1(a), with an appropriate bias voltage and a sufficient concentration of free ions, a measurable current proportional to the contact area should occur. The current density is mainly determined by the overpotential across an electric double layer formed at the contact area [15]. As the tip is being pulled away, the contact area will decrease so that the current becomes smaller until the contact is broken off. Before the contact breaks, there is a column of water, hereinafter called a bridge, between the tip and the water film. When the constant-current mode is used, the feedback mechanism will keep the tip-surface distance constant, provided the thickness of the water film is approximately uniform. Therefore, the altitude of the tip provides information about the surface topology of the specimen. Such an imaging node does not pose any limitation on the specimen thickness as long as the water film provides a closed circuit for ion conduction. To validate this idea, we analyze the formation of the water bridge, assuming that the water film is extended to and always connected with an infinitely large reservoir to ensure a moist surface on the specimen [16].

The scenario described above seems so obvious and logical that one might think of it as no more than a daily observation. However, this expectation is incorrect. To illustrate this point, we first apply the macroscopic theory.

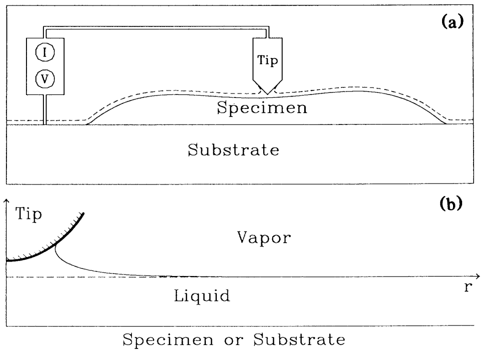

FIG. 1. (a) The experimental chamber with controlled temperature and humidity. (b) Sketch of the liquid-vapor interface. The sign of the principal radius of curvature is defined as negative if the corresponding curvature vector is pointing to the vapor phase.

Let the liquid-vapor interface far away from the bridge be located at $z=0$. Then, using Laplace's formula [17], cf. Fig. 1(b), one easily obtains $\sigma(T)/r_m(r,z)+\rho gz=0$, where $\sigma(T)$ is the surface tension of the liquid-vapor interface, whose mean radius of curvature is $r_m(r,z)$, ρ is the mass density of the liquid, and g is the acceleration of gravity. Assuming an azimuthal symmetry, we obtain the equation for the interface profile

$$1+\hat{r}'^2-\hat{r}\hat{r}''+\beta\hat{z}\hat{r}(1+\hat{r}'^2)^{3/2}=0\,, \tag{1}$$

where $\beta=\rho gR^2/\sigma(T)$, $\hat{z}=z/R$, $\hat{r}=r/R$, $\hat{r}'=d\hat{r}/d\hat{z}$, $\hat{r}''=d^2\hat{r}/d\hat{z}^2$, and R is a length scale. This equation is, in spirit, similar to that of Bashforth and Adams [18,19]. The dimensionless factor β represents the competition between the gravitational potential energy and the surface free energy. Larger values of β mean that gravitation dominates. The profile of the bridge near the tip, if formed, is bent, resulting in a smaller lateral dimension of the bridge compared to that of the tip. Smaller values of β do not allow the profile to be bent too much because it would increase surface energy without being balanced by gravitational energy reduction. As a result, for a given contact angle on the tip, the interface will climb up so that the bridge becomes much larger in size than the tip [20]. For water at room temperature ($T=295$ K), if $R=1$ cm, then $\beta=13.5$ and a bridge with a smaller size (around 0.7 cm) is formed with the contact area varying with the tip-surface distance. However, for $R<1$ μm, $\beta<1.35\times10^{-7}$, and the climbing phenomenon dominates. The tip would be immersed into water completely and the contact area would be insensitive to the tip-surface distance. If this description were correct, the proposed imaging mode would be impossible. However, in the above analysis, two factors in addition to gravitation and surface tension have been overlooked. First, the material-dependent long-range interactions must be considered. These interactions are responsible for the disjoining pressure [16,21] which measures the pressure discontinuity across the interface. Taking this factor into account, we find that the conserved quantity on the interface is $\sigma(T)/r_m(r,z)+\rho gz-P_d(z+t)$, where $P_d(z+t)$ is the disjoining pressure at level z. Therefore, we obtain

$$\sigma(T)/r_m(r,z)+\rho gz=P_d(z+t)-P_d(t)\,. \tag{2a}$$

The second factor that must be considered is the thermal equilibrium. If the vapor is assumed to be an ideal gas, then far away from the bridge we have

$$P_d(t)=-\mu_a(t)=\rho k_BT\ln(1/H)/m\,, \tag{2b}$$

where $\mu_a(t)$ is the excess chemical-potential density for the adsorbates, k_B the Boltzmann constant, m the molecular mass, and $H=P_g/P_s$ the relative humidity with P_g and P_s the vapor pressure and the saturated vapor pressure, respectively. The theory of long-wavelength electromagnetic fluctuation is well established [22]. For the thin-film case (a few hundred Å or less), where retardation [23] is unimportant, we have [22] $P_d(t)=a/t^3$, where the coefficient a depends upon the materials involved and can be determined either theoretically or experimentally [19,22,24]. Using the same definitions as in Eq. (1), we obtain

$$1+\hat{r}'^2-\hat{r}\hat{r}''+\{\beta\hat{z}+\gamma[1-(1+\hat{z})^{-3}]\}\hat{r}(1+\hat{r}'^2)^{3/2}=0\,, \tag{3}$$

where $R=t$ and

$$\gamma=a/t^2\sigma(T)=[\rho k_BT\ln(1/H)/am]^{2/3}a/\sigma(T)\,.$$

Note that this equation depends only on two dimensionless parameters β and γ, which in turn depend on temperature and humidity. It is easy to show that $\beta\ll\gamma$ at moderate humidity, so that the gravitation can be omitted. The γ term in Eq. (3) behaves similarly to the β term in Eq. (1) for small $\hat{z}$. Thus we expect that Eq. (3) describes a qualitatively similar behavior of the interface at microscopic scales as that described by Eq. (1) at macroscopic scales. Unlike β in Eq. (1), γ is very sensitive to temperature and humidity. By increasing (decreasing) humidity or decreasing (increasing) temperature, γ is decreased (increased) and t is increased (decreased). Thus the surface is more (less) likely to climb up. In the limit $H\rightarrow100\%$, the microscopic theory reduces to the macroscopic theory.

In solving for the shape of the bridge, the profile of the interface needs to pass a turning point where $\hat{r}'=0$. Below this point, we may neglect the influence of the tip and apply Eq. (3). Above this point, the profile will be affected primarily by the tip. Without going into details about the tip geometry and other tip properties, we may assume that the tip has a very small radius of curvature so that the van der Waals forces and possible double-layer repulsive forces [25] have much shorter range than that in a flat geometry. Therefore, as an approximation, we neglect those forces and the influence of the specimen below. With the assumed azimuthal symmetry, the profile of the bridge above the turning point is simply catenoid [17]. Two solutions will merge at the turning point. The equation governing the bridge profile is simplified to

$$1+\hat{r}'^2-\hat{r}\hat{r}''=\begin{cases}0, & \hat{r}'>0,\\ -\gamma\hat{r}[1-(1+\hat{z})^{-3}](1+\hat{r}'^2)^{3/2}, & \hat{r}'<0.\end{cases} \tag{4}$$

For simplicity, we further assume that the tip is conical with a smooth surface. Since contact angles are usually measured on a flat surface, it is by no means clear whether they can be applied to the tip where the mean radius of curvature is very small. Nonetheless, by assuming that the contact angle is small [26] and is a constant on the tip, we obtain the boundary conditions necessary for solving Eq. (4): $\hat{r}|_{z=a}=(a-D)\cot(\theta)/t$ and $\hat{r}'|_{z=a}$

$=\tan(\theta-\phi_0)$, where a is the position of the contact circle, D the distance of the tip from the surface of the water film, θ the half angle of the conical tip, and ϕ_0 the small contact angle. Figure 2 shows some numerical solutions of Eq. (4) with a conical tip ($\theta=45°$). The contact areas for different θ are obtained numerically, as shown in the inset of Fig. 2. For a given distance, the contact area increases with θ. For a given θ (a tip property), the contact area (hence the current) varies sensitively with the distance. This provides the necessary feedback signal to control the tip position.

The above analysis has implicitly assumed that the tip surface is dry (meaning that the water adsorption is so light that ion conduction is prohibited [27]) around the contact area. This is a *necessary* condition for this imaging mode to be successful since it requires a sensitive relationship between a *finite* contact area and the tip-surface distance. Experiments have shown that uncleaned surfaces generally adsorb water molecules much more heavily than cleaned surfaces do [24]. For biological materials, most specimen surfaces belong to the "uncleaned" category, while tip materials (e.g., platinum) are relatively easy to clean (by high-temperature degassing [24], for instance) and thus are not easy to wet. The often used tip conditioning technique, such as large current at high bias, may also serve the purpose of surface cleaning. In addition, a tip with a small radius of curvature is even more difficult to wet. Although it is beyond the scope of this Letter to discuss this point in detail [28], a simple analysis shows that for a sphere at room temperature there exists a critical radius $r_c \approx 13.8\ln(1/H)$ (nm) below which the sphere is dry. Therefore, we conclude that the apical region of the tip should be dry even if its root is wet. When humidity is high, there is a possibility that the critical radius of curvature r_c becomes so small that the water film on the root of the tip merges with the bridge. This makes the contact area (hence the current) very large and insensitive to the tip-specimen distance. The tip will be pulled back to reduce the current until the bridge breaks and the current subsequently drops to zero. Then the tip will move forward to reestablish the current. This action forces the tip to oscillate.

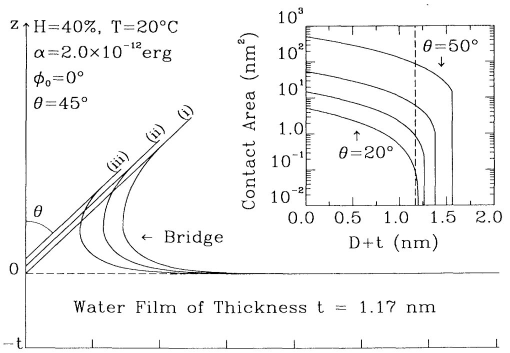

FIG. 2. Some solutions of Eq. (4) with different distances: (i) $D=0$, (ii) $D=1.2$ Å, and (iii) $D=2.4$ Å. Inset: The distance dependence of contact area. The half conical angles are, consecutively, $\theta=20°$, 30°, 40°, and 50°. The dashed line indicates the position of the liquid-vapor interface in the absence of the tip.

Once a bridge is formed, a measurable current can be obtained by applying a reasonable bias voltage. A necessary condition is of course that the water film continuously covers the surfaces of the specimen and the conducting substrate (at least partially). The conductivity of water, depending on the concentration of free ions, is in the range of 10^{-8}–10^{-7} Ω^{-1} cm^{-1}. The resistance of the water film and the bridge will significantly reduce the overpotential across the electric double layer at the tip-water interface from the overall bias potential. Therefore a higher bias voltage is normally needed in order to obtain a sufficient current. For a contact area of 10 nm in diameter, 1-pA current can be obtained for a current density of about 1.3 A/cm^2, achievable for a moderate overpotential.

Obviously, it is advantageous to use low humidity to improve resolution for a thinner water film. But too low a humidity may prevent the formation of a continuous film so that the ionic conduction may be disrupted. Too high a humidity is not only a bad choice for high resolution, but also causes the tip to oscillate as predicted previously. There are two types of specimens in terms of scales of their corrugation. The class with large-scale corrugation can be covered with an almost uniform water film. Thus a near replica of the surface topology can be obtained. The class with small-scale corrugation, however, will be covered with a film which varies in thickness. The crossover scale R_c is determined by $R_c = m\sigma/\rho k_B T\ln(1/H)$. For water around room temperature, $R_c = 1.56\times10^3/T \times\ln(1/H)$ (Å). For $T=295$ K, $H=40\%$, we find $R_c \approx 5.8$ Å. Therefore, the image resolution should be determined by the lateral dimension of the bridge for most cases. As shown in Fig. 2, this dimension is 1–2 nm at best for the given humidity and temperature.

In conclusion, we have presented a new principle of operating the STM for topological imaging of nonconductive materials. One advantage of this imaging mode is that it exerts minimal force on the specimen surface. According to our calculation, the resolution is on the order of nanometers, strongly dependent on the humidity and temperature, and independent of specimen thickness. Although the above analysis is based on ideal surfaces, the concept and the qualitative features of the model should be valid in real systems. In fact, we believe that this imaging mode has already been realized in at least one experiment by Guckenberger *et al.* [29]. In their experiment, a bacterial surface protein, HPI layer, was imaged by STM. It was reported that the image can only be obtained at mid-range humidity (30%–45%) at high bias voltage (up to 10 V) using constant-current mode with extremely low current (lower than 0.5 pA). At higher humidities, the tip began to oscillate, while at lower humidities no images were obtained. 3-nm pores were clearly resolved, even when several HPI layers piled up to a few hundred angstroms. The resolution did not depend on specimen thickness. All the above observations are consistent with the theoretical model. In particular, the experimental parameters are very close to the theoretical predictions. At nanometer resolution, this mode of operation will be very useful in biological research of surface structures.

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