

Ultralarge atomically flat template-stripped Au surfaces for scanning probe microscopy

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We have devised a method to produce gold films with ultralarge atomically flat areas for use in scanning probe microscopy. A mean roughness of 2 Å for areas of 2.25 μm², and about 3 Å for 25 μm², can be easily produced. The method is based on: (i) epitaxial growth of gold on mica; gold is thermally deposited onto freshly cleaved mica sheets, (ii) glueing the fresh gold surface to a piece of Si wafer, and (iii) chemical or consecutive mechanical stripping of the mica down to the freshly appearing gold surface (i.e., template-stripped gold). We present a detailed STM and AFM roughness study of these gold films.

1. Introduction

Scanning tunneling microscopy (STM), atomic force microscopy (AFM), and related techniques are increasingly important tools for investigating the surface topography of immobilized molecules or biological structures. One of the major advantages of these methods is that they allow measurement of surface features in air or in liquids at room temperature [1], so that dynamic events involving biological macromolecules or organelles should be in principle observable in real time under conditions approaching “physiological”.

Physical adsorption provides in general insufficient immobilization at the liquid/substrate interface and may lead to denaturation of samples. Therefore, covalent linking (or bonding of comparable strength) to a surface is in principle preferable. The substrate should be stable and chemically inert i.e. against oxygen, yet accessible directly to biological samples, or to chemisorbed bifunctional monolayers (which should lead to surfaces with specific reactivity onto which to

anchor biological or organic polymers and/or organelles). It is also mandatory that the substrate should have very large and quite flat areas, in order to easily distinguish the deposited specimen from the substrate with scanning probe microscopy (SPM). One of the first substrates tested with biological macromolecules was highly oriented pyrolytic graphite (HOPG). Although HOPG meets some of the requirements indicated above, surface features of “clean” HOPG images are often difficult to interpret, and may even mimic biological macromolecules such as DNA [2]. Also, the forces binding proteins or nucleic acids to HOPG are likely to be fairly weak, and may not be stable in the presence of solutes, which is important if dynamic interactions under “native” conditions are to be investigated, e.g. in buffers. Finally, the adsorption of proteins to HOPG bears the potential danger of denaturation or deformation of protein structures.

Gold is a popular substrate. It is stable against oxygen, can be easily prepared by vapor-deposition, and can bind with high affinity organic thiols or bifunctional disulfides such as gold(I)alkanethiolates [RS-Au(I)] (see, e.g. refs. [3–11]) also capable of forming self-assembled monolayers. The epitaxial growth of fcc metals on mica as

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a substrate is well known [12], and Au thin films which form with (111) orientation on the (001) cleavage planes of mica have been extensively studied. Chidsey et al. [13] and Putnam et al. [14], reported in detail the effect of deposition temperature, deposition rate, and film thickness on the flatness of Au films on mica, and the process was optimized by De Rose et al. [15].

Au surfaces prepared by the above procedures suffer from a number of imperfections (see, e.g., ref. [16]). The terraces obtained by vaporizing Au on mica at optimal temperature, i.e. 300–500°C, which is the most widespread technique, have varying sizes and are surrounded by unpredictable and rough topography. Indeed with vaporization at constant temperature it is impossible to avoid the formation of deep depressions [13]. Hence these are useful only for macromolecules which are smaller than the terraces themselves. Other Au surfaces (e.g., single crystals, annealed gold balls, etc.) suffer likewise from various limitations, such as high cost or lengthy preparation procedures [16]. Finally, all Au surfaces described thus far have to be prepared immediately before use, which is time-consuming for routine use.

We have thus investigated the possibility of developing a simple procedure to reproducibly prepare ultralarge, atomically flat gold areas. Such a procedure is described in this paper. Within a surface of several mm², random samples of 2.25 μm² exhibit a mean roughness of less than 2 Å, and samples of 25 μm² one of less than 3 Å. An additional practical advantage is that our gold surfaces can be quickly obtained from their immediate precursors, which are stable and can be prepared and stored in bulk.

2. Experimental

2.1. Materials and instrumentation

Instruments used include a vacuum coating system BAE 370 from Bal-Tec (Liechtenstein) with integrated quartz crystal deposition controller Inficon XTC/2 from Leybold (Switzerland). A few experiments were done on a vacuum

coating system PST 170 E also from Bal-Tec (Liechtenstein). Scanning tunneling microscopy and atomic force microscopy were carried out on a Nanoscope III from Digital Instruments Inc. (CA, USA). We purchased the Nanoprobe calibration standard (Si pyramids with 200 nm period) and Si cantilever from LOT (Germany). An Alpha-step 200 profilometer Tencor was from Polyscience (Switzerland). Gold (99.999%) was purchased from Cendres & Metaux SA (Switzerland) and ruby muscovite mica from Bal-Tec (Liechtenstein), Goodfellow (UK) or Plano (Germany). Monocrystalline Si(100) wafers were from Faselec (Switzerland). The low-viscosity epoxy glue epo-tek[®] No. 377 was from Polyscience (Switzerland). All chemicals and solvents were commercial grades of highest purity.

2.2. Preparation of gold films

Clear ruby muscovite mica sheets (35 cm²) were freshly cleaved with a glaucoma knife and immediately placed cleaved side down into the molybdenum sample holder of the vacuum system BAE 370. The resistively heated tungsten boat for thermal metal evaporation was 25 cm directly below the mica substrate. The mica sheet can be radiatively heated in situ from the rear through the molybdenum sample holder, with temperature feedback control through a thermocouple. Before the evaporation of Au, the mica sheet was first heated for more than 20 h at 300°C at a pressure of less than 1.33×10^{-3} Pa (10^{-5} Torr) and then equilibrated 2 h at the temperature chosen for the evaporation, i.e., 20–25, 100, 200, 300, 400, 500 or 600°C. (The temperatures finally adopted for routine work were between 25 and 300°C; see section 3.)

Evaporation of gold was carried out at a pressure of less than 2.66×10^{-4} Pa (2×10^{-6} Torr). Evaporation rate, which was typically 1 Å/s, and film thickness (~ 2000 Å) were monitored by a quartz crystal thickness controller and checked with a profilometer. Five minutes after closing the shutter, the samples were cooled (10°C/min) to room temperature. After removal from the evaporation chamber it is not necessary to store the gold films under special protective conditions.

2.3. Stripping

Gold-deposited mica sheets were cut into small pieces ($\sim 1 \text{ cm}^2$) and glued gold face down onto Si(100) wafer pieces using a low-viscosity epoxy glue. After 1 h at 150°C , the glue had reached an adequate hardness. A surplus of epoxy glue should be avoided. This mica/gold/Si “sandwich” can be stored as a “stripping precursor” at least up to several months without loss of quality.

2.3.1. Method A: mechanical stripping

The mica (with typical thickness of about $40 \mu\text{m}$) was carefully stripped off in successive layers using an adhesive tape. In order to follow the appearance of the fresh gold surface, after each stripping the conductivity of the surface was checked and the surface examined by light microscopy. This procedure requires some practical experience, but is especially rapid. Gold films with remaining mica islands were discarded ($< 5\%$).

2.3.2. Method B: chemical stripping

We had seen in preliminary experiments that soaking the mica/gold/Si “sandwiches” in various solvents lead to detachment of the complete mica sheet from gold/Si (“chemically stripped gold surface”) without undue effect on the epoxy glue. We therefore tested 30 different solvents with a wide range of polarities and selectivity.

We identified 7 solvents as suitable for chemical stripping: tetrahydrofuran (THF), N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), pyridine, 2-picoline, 3-picoline, and 1,4-dioxane. Snyder [17] has presented a detailed study of solvent properties, and classified the solvents tested into 8 groups according to their “ x_i -values”, which represent the ability of the solvent to engage in hydrogen bonding or dipole interactions (i.e., x_e : proton acceptor; x_d : proton donor; x_n : strong dipole). All the solvents found useful for chemical stripping belong to group III of Snyder’s x_i -triangle or are very close to it (dioxane), and show a ratio of x_e to x_d and of x_n to x_d between 1.7 and 1.9. It is important to note that these solvents have been identified with reference to the particular epoxy glue we used.

Other epoxy glues may not withstand the chemical stripping with the solvents indicated above.

Routinely, the mica/gold/Si “sandwiches” are soaked in solvent (preferably THF) at room temperature with gentle shaking until the mica sheet separates from the gold surface. This takes some five minutes with THF and approximately half an hour with the other solvents mentioned above. Immediately thereafter the chemically stripped fresh gold surfaces are rinsed with ethanol and dried in vacuum. Removal of mica with hydrofluoric acid is not recommended, as the epoxy glue is not stable in this solution.

2.4. SPM measurements

The measurements were performed under ambient conditions on a commercially available scanning tunneling and atomic force microscope. All STM data were acquired with Pt/Ir (90:10) tips, which were made by electroetching of 0.25 mm wire in 2.6M KCl, 0.4N HCl (cooled to -12°C), at an AC voltage of 12 down to 2 V [18]. Tunneling current (I_{tun}) was 1 nA and tip bias voltage (V_{bias}) was negative 200 mV for large scans, and 2–6 nA and negative 5–10 mV for atomic resolution. STM images were taken in the constant-current imaging mode. For AFM we used various monocrystalline silicon cantilevers with integrated silicon tips, with spring constants ranging from 0.08 to 0.17 N/m [19,20]. Image acquisition in the constant-force imaging mode was done in air and in various liquids (such as ethanol or “nanopure” water) at room temperature with repulsive forces of the order of 1–5 nN. Our scanners were calibrated in all dimensions using a Nanoprobe calibration standard (Si/200 nm period). The known lattice constants were observed on HOPG and mica; z-calibration was confirmed by measurement of Au(111) step heights. All images shown in this paper are based on unfiltered data.

3. Results

Vapor deposited gold films were first prepared as described by others [12,13,15,16,21–24]. In or-

der to achieve optimal size and flatness of the terraces, we prepared a series of gold films at various deposition temperatures. The topography of the gold surface facing the evaporation chamber (the "outer" surface) showed the well-known behaviour, i.e. coalescence between 250 and 300°C and atomically flat regions of several hundred Å in diameter after annealing at 500°C, thus confirming previous observations. We have now subjected these very same specimens to our stripping method (as described in section 2) to obtain the surfaces described below.

We generated several hundred STM and AFM images and carried out the corresponding roughness calculations for both the "outer" surface and the gold surface originally facing the mica ("template-stripped"). The tip quality (e.g. tip radius, cone angle) was investigated by SEM and in the case of AFM with a pyramidal Si standard. Only tips yielding atomic resolution in preliminary experiments on gold or freshly cleaved mica or HOPG surfaces were used in our studies.

Each sample was statistically probed, in that within an area of 36 mm² three scans of 25 μm² and nine scans of 2.25 μm² were made and the roughness calculated with the analytical features of the Nanoscope III. Table 1 summarizes the

mean roughness R_a (nm) of samples prepared at deposition temperatures from 20 to 600°C, examined in air and mechanically stripped (method A). Very similar R_a values were obtained with stripping method B and SPM measurements in liquids (data not shown). The template-stripped gold surfaces show excellent flatness over large areas when prepared below 300°C. The roughness hardly increases from 20 up to 300°C, rising significantly at 400°C and above. As shown in table 1, the roughness of the template-stripped surface is approximately one order of magnitude better than that of the "outer" surface in the same sample. The mean roughness of template-stripped gold surface deposited between 20 and 25°C, as determined by STM, was approximately 2 Å for areas of 2.25 μm², and approximately 3 Å for areas of 25 μm². A deposition temperature of 400°C causes a 2-fold increase in mean roughness, and a temperature of 600°C a 14-fold increase. Analogous deposition experiments on an older vacuum coating system (PST 170 E), with a vacuum of approximately 1.33×10^{-3} Pa (10^{-5} Torr), lead to similar results.

Fig. 1A shows a representative STM image of a template-stripped gold film (deposition temperature 25°C), and the corresponding roughness

Table 1

Mean roughness values [$R_a(\text{nm}) \pm \sigma$] ^{a)} of the outer and the template-stripped gold surfaces of the same specimens

Sample temperature (°C)	Outer surfaces		Template-stripped surfaces							
	AFM		AFM				STM			
	25.0 μm ²		25.0 μm ²		2.25 μm ²		25.0 μm ²		2.25 μm ²	
600	15.93	± 1.65	3.29	± 0.50	3.02	± 0.70	2.67	± 0.30	2.80	± 0.76
500	4.03	± 1.25	2.11	± 0.17	1.14	± 0.21	1.23	± 0.17	1.09	± 0.27
400	2.10	± 0.21	1.04	± 0.31	1.33	± 0.18	0.63	± 0.19	0.39	± 0.02
300	1.00	± 0.14	1.14	± 0.01	1.01	± 0.06	0.33	± 0.02	0.27	± 0.01
200	7.33	± 0.09	1.09	± 0.25	0.84	± 0.05	0.36	± 0.03	0.29	± 0.02
100	4.71	± 0.09	0.59	± 0.11	0.49	± 0.03	0.40	± 0.01	0.37	± 0.01
20–25	3.36	± 0.04	0.32	± 0.01	0.28	± 0.01	0.25	± 0.01	0.20	± 0.01

$$a) R_a = \frac{1}{L_x L_y} \int_0^{L_x} \int_0^{L_y} |f(x, y)| dx dy;$$

$$\sigma = \sqrt{\frac{n \sum R_a^2 - (\sum R_a)^2}{n(n-1)}} \quad (25.0 \mu\text{m}^2 \rightarrow n = 3; 2.25 \mu\text{m}^2 \rightarrow n = 9);$$

L_x and L_y are the dimensions of the surface and $f(x, y)$ is the surface relative to a calculated flat plane based upon the surface data that has equal volume above and below the plane.

data along the cross section indicated (fig. 1B). The mean roughness R_a of the whole image (x -range $5\ \mu\text{m}$) is $2.0\ \text{\AA}$; that of the $7\ \mu\text{m}$ long

cross section is $1.8\ \text{\AA}$, that of the box (x -range $1\ \mu\text{m}$) is $1.8\ \text{\AA}$. These surfaces show by STM the usual hexagonal (111)-orientation, with lattice

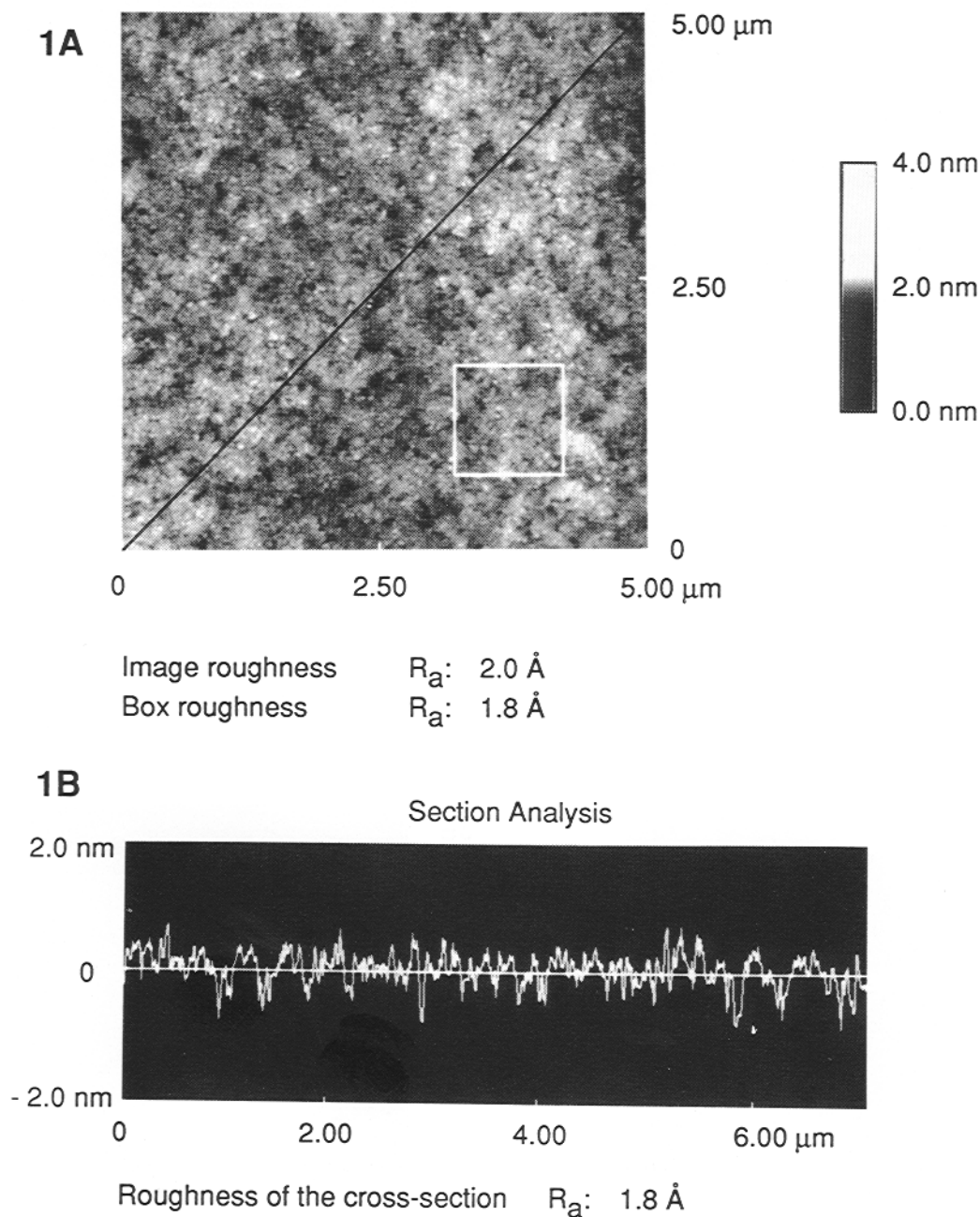


Fig. 1. (A) STM image of a template-stripped gold surface (deposition temperature 20°C). Box size $1\ \mu\text{m} \times 1\ \mu\text{m}$. (B) Cross-section of the same image with diameter in length of $7.07\ \mu\text{m}$. Note that all pictures reported were obtained with a steep contrast gradient (see the z -range bars in this and in the following figures).

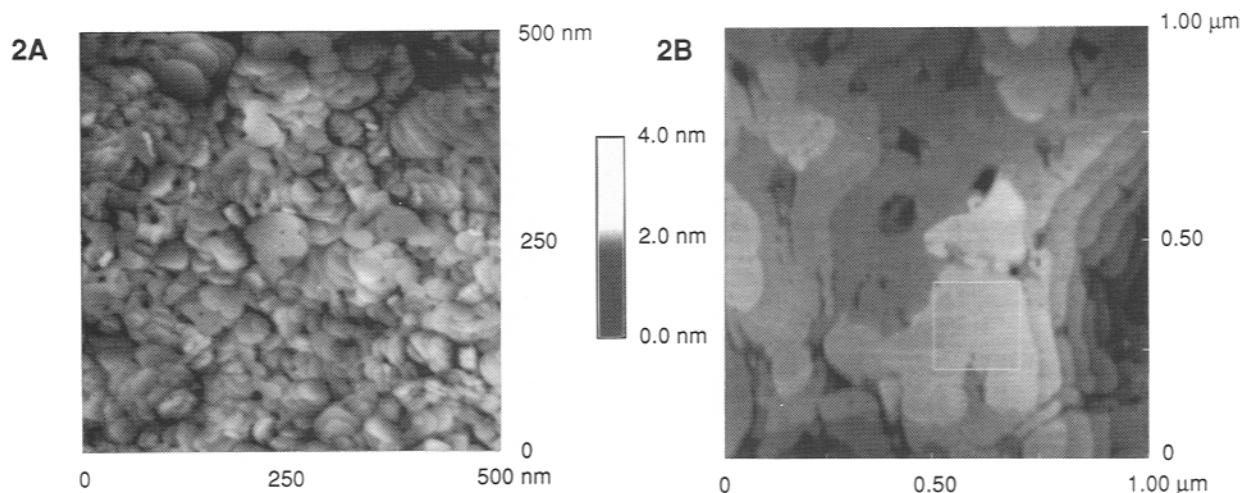


Fig. 2. (A) STM image of a template-stripped gold surface (deposition temperature 22°C). The z-range bar is identical for A and B. (B) STM image of a template-stripped gold surface (deposition temperature 300°C). Box size 200 nm $\times$ 200 nm with $R_a = 0.03$ nm.

constants of 2.88 Å independent of the deposition temperature (data not shown).

Figs. 2A and 2B show template-stripped gold surfaces prepared at deposition temperatures of 20°C (2A) and 300°C (2B). The surfaces exhibit flat grains that differ in height by only a few monatomic steps (figs. 2A and 2B), in contrast to those in the outer Au surface. This property leads to the extremely low roughness extending over

very large areas. When deposited at 20°C, the film is initiated by the nucleation of small flat grains, with average diameters of approximately 50 nm (fig. 2A). Heating the mica to 300°C during the deposition causes a clear increase in terrace size, with atomically flat areas of approximately 500 nm average diameter. This can be attributed to more rapid diffusion of Au on the surface of the mica [22]. As mentioned above, there is no

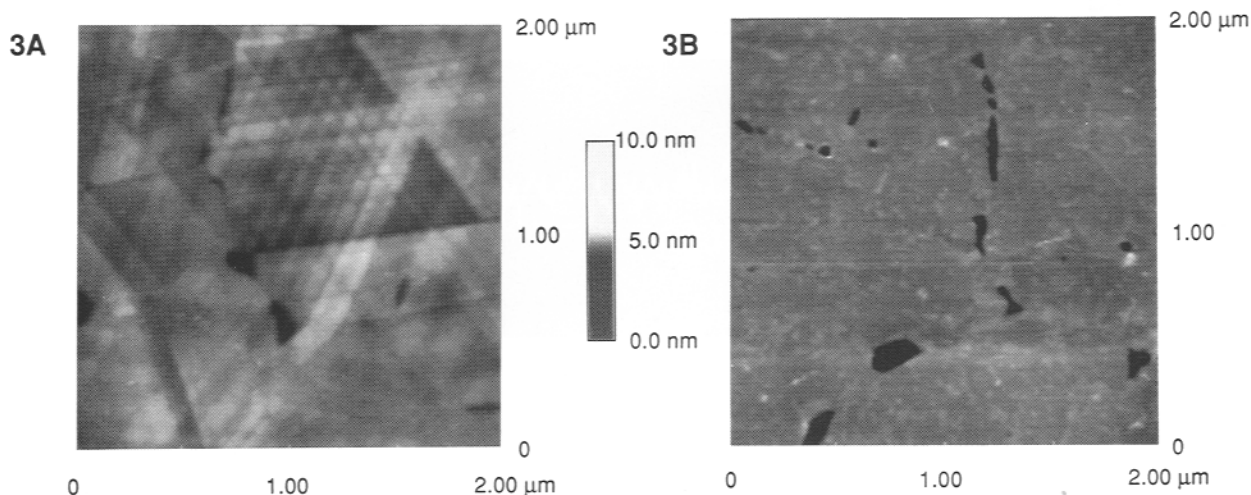


Fig. 3. STM (A) and AFM (B) images of a template-stripped gold surface (deposition temperature 500°C). The z-range bar is identical for A and B.

significant increase in R_a of the template-stripped surface as the deposition temperature is raised from 20 to 300°C (see table 1). The choice between these temperatures depends on size of the object to be eventually placed on the gold surface, and on the resolution aimed at.

The mean roughness values in table 1 were obtained from x -ranges as long as 5 μm encompassing some hundred atomically flat terraces. For the template-stripped gold surfaces, the z -levels of the terraces are within approximately 1.5 nm. The problem of identifying macromolecular or supramolecular structures, with an extension in the z -direction of several nm, on a suitably large terrace would therefore not arise when deposited on the template-stripped surface. As to the "outer" surfaces, the roughness of the individual terraces (with diameters of a few hundred nanometers) is comparable to that of similar surfaces investigated by previous workers; since the terraces differ greatly in height, the mean roughness of *large* images is far worse than that of the template-stripped side.

Figs. 3A and 3B represent an STM and an AFM image, respectively, of template-stripped gold films deposited at 500°C. Triangular platelets (visible in the STM image) often appear when measuring (111)-oriented deposits [15]; such features were not seen in the AFM images.

In order to minimize the contamination of the deposited gold by carbon, water, etc., the mica support was preheated at 300°C for several hours. The contamination, e.g., arising from diffusion of ions from the mica into the deposited Au surface, is not relevant when biological specimens are to be examined. Therefore we have not carried out an Auger electron spectroscopy (AES) and/or electron spectroscopy for chemical analysis (ESCA) of our Au surfaces.

4. Conclusions

In summary, the template-stripped gold surfaces prepared by our stripping procedures seem to be superior to other Au(111) surfaces in several respects: (i) they are very large, with very low roughness over extended areas (typically of 2 Å in

2.25 μm^2 and of 3 Å in 25 μm^2), (ii) they are easy to prepare, and (iii) they can be made immediately before use from the gold-covered mica specimens. These are stable for months and can thus be prepared and stored in bulk. Images such as that in fig. 1, which were obtained routinely, and the data in table 1, make our template-stripped surfaces highly suitable gold substrates for scanning probe microscopy. Indeed, our surfaces will be classified as belonging to the most "desired zone" in fig. 4 of Clemmer and Beebe's review [16].

Finally, our template-stripped gold surfaces are well-characterized potential alternative substrates for nanolithography with scanning tunneling microscopy [25–28].

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