Meniscus Force Nanografting: Nanoscopic Patterning of DNA

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Thiol-modified DNA is patterned with an atomic force microscope (AFM) on a resist-covered gold surface with line widths as small as 15 nm at speeds as high as 320 μm/s. In meniscus force nanografting, a drop of patterning solution simultaneously wets a hydrophilic resist on gold and an AFM tip. The surface tension supplies enough normal force for the tip to selectively remove the resist molecules and allow the DNA to attach to the gold surface. Different DNA-derivatized nanostructures can then hybridize to the complementary, surface-bound DNA in a demonstration of surface initiated orthogonal self-assembly.

In a wide variety of biotech, chemical, and nanoengineering applications, reactions are initiated on a surface. It is often of great advantage to conduct many investigations simultaneously in the same environment in disciplines such as biosensing, chemosensing, genetic tests, and combinatorial investigations meant to create new products, find new ways to produce these products, or optimize processes that are already known. To this end it is very desirable to be able to permanently pattern substances to a surface with great precision and speed, so as to increase the number of experimental variations possible and decrease the amount of materials necessary. The ability to pattern DNA is of particular interest because of the complete specificity in binding partners it demonstrates during hybridization. Besides the advances it would make in gene detection, the nanoscopic control of DNA patterning would allow one to independently assemble different (DNA-derivatized) nanostructures to the surface in a process called surface initiated orthogonal self-assembly.[1] This ability of controlled assembly from a surface holds great promise in fields such as molecular electronics, micro- and nanofabrication, and nanotechnology. DNA arrays have been produced with spot sizes as small as 20 μm by means of a photolithographic process and are detected by laser fluorescence[2]—the precision of both processes being limited by the wavelength of light. While adequate for genetic sequencing, this resolution is far below the nanometer precision required for arrays of molecular electronic elements, for example. This paper presents meniscus force nanografting (MFN) as a fast, convenient, and versatile way to nanoscopically pattern molecules and in particular demonstrates the capability of patterning DNA with nanometer precision. We furthermore detect the nanostructures of DNA with similar accuracy with an AFM (atomic force microscope) or SEM (scanning electron microscope) after allowing DNA-derivatized nanoparticles to hybridize to the surface-bound DNA.

Lithographic processes are at the heart of nanotechnology and initiate the process of computer chip manufacturing. Traditionally, lithography has consisted of "negative" or "subtractive" methods whereby material is selectively removed from a surface—either by an electron beam or the chemical etching of a substrate that is selectively protected by a resistive film. Recent progress in "positive" or "additive" lithography methods—whereby patterns are formed by *adding* material to a substrate—has the potential to expand nanolithography to include the patterning of chemical and biological elements. In the effort to bind molecules to a surface, the thiol−gold adsorbate−substrate system[3] has been the most popular because of gold's inert behavior to most substances except sulfur. The success of microcontact printing[4] to directly apply thiol-modified molecules on a gold surface with an elastomer stamp initially set the scale for molecular patterning capability at hundreds of nanometers. Recent use of scanning probe microscopes such as AFMs and STMs has brought much higher resolution to the patterning process.[4,5] The invention of DPN[6] (dip-pen nanolithography) brought the length scale of patterning down to 15 nm by virtue of direct molecular diffusion of the appropriate substance (such as octadecanethiol or mercaptohexadecanoic acid) from the AFM tip to the substrate but is limited in both writing speed and variety of patternable materials by the natural diffusion rates of the patterning substance. The invention of an indirect lithography called nanografting by Liu et al.[7] greatly expands the repertoire of patternable substances—from small chain hydrocarbons to proteins[8]—but is also a slow process (~0.1 μm/s) and must be done in a fluid cell. In nanografting, the gold surface is passivated with a resist (such as a monolayer of alkanethiol) and placed in an AFM fluid cell under a solution of the material to be patterned. At high contact force (10−20 nN) the resist molecules are removed by the AFM tip, exposing the undisturbed, bare gold surface, onto which the solution-born patterning material can then adsorb resulting in lines as thin as 10 nm. More recently,

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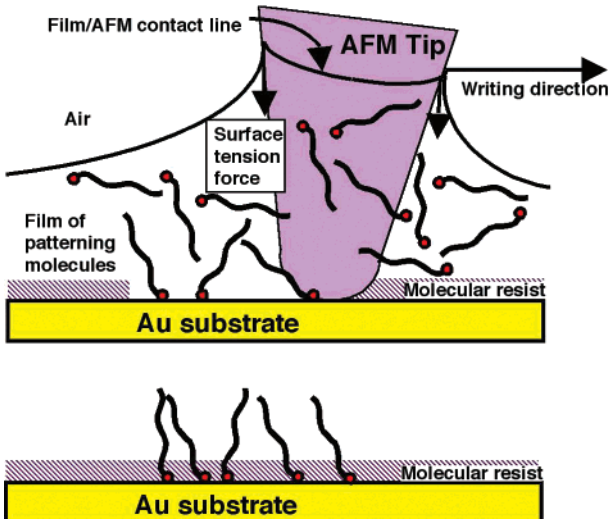

Figure 1. MFN molecular patterning. A thin film of patterning molecules in solution is prevented from bonding to a gold surface by a molecular resist. The resist is selectively removed by the sheer force of an AFM tip (above), allowing patterning molecules to have access to the gold surface and bond. The normal force between the AFM tip and the substrate is provided by the surface tension of the thin film of patterning fluid. After patterning, the resist can be applied again to passivate the substrate in the freshly patterned areas in preparation for subsequent MFN patterning.

Liu et al. have nanografted in open air without a fluid cell by delivering the patterning molecules from a *coated* AFM tip through a resist with high contact force, in what could be called dip-pen nanografting.[9] A positive lithography similar to nanografting called micromachining[10] conducted prior to Liu's work, resulted in line widths as small as 100 nm. Micromachining, which makes use of millinewton forces applied on a carbon fiber (instead of an AFM tip), results in indentation of the gold surface tens of nanometers deep. More recently, a nanografting technique has been developed which makes use of a scanning tunneling microscopy (STM) tip to selectively destroy a monolayer resist at speeds of 15 μm/s, resulting in lines as thin as 30 nm.[11]

Meniscus Force Nanografting

We present here another variation of additive, indirect scanning probe lithography called meniscus force nanografting (MFN), whereby the patterning material is applied as a small drop on a resist-covered substrate, and an AFM tip selectively penetrates the resist, delivering the patterning material to the gold surface. Traditionally, the normal force between the AFM tip and substrate necessary to displace the resist molecules is supplied by the flexing of the AFM cantilever and is kept constant by AFM feedback.[12] We have found that the surface tension of a liquid drop itself can supply the constant force necessary to displace the resist molecules (Figure 1), eliminating the need for AFM feedback control. Furthermore, the force supplied by the meniscus is high enough for the AFM tip to flatten the surface of polycrystalline gold as well as strip it of any adsorbate molecules. Thus, MFN patterning can proceed extraordinarily fast. We have been able to pattern lines as fast as 320 μm/s (more than

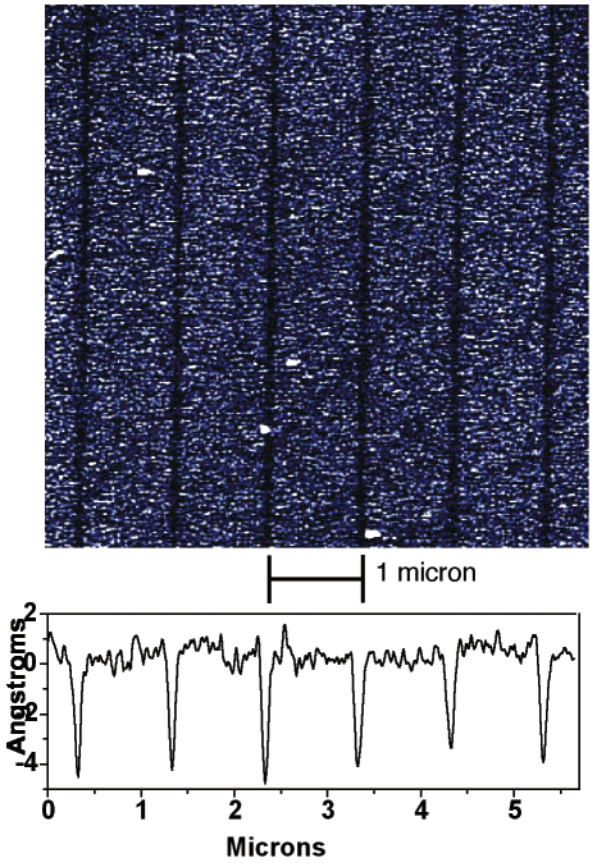

Figure 2. Nanoindentation. An AFM topograph (above) of a gold surface immediately after the MFN patterning of vertical lines. Below is a horizontal cross-sectional average of the topograph. The high perpendicular force possible during MFN patterning can result in flattening of the gold surface. The vertical lines shown have an average depth of 5 Å. Diagonal lines directed up to the right are also barely discernible, having an average depth of 1 Å. Diagonal lines were also patterned up to the left and are not discernible. The variation in depth is the result of variation in normal force between the AFM tip and the gold surface caused by differences in the size of the drop of DNA solution that is attached between the resist-covered gold surface and the hydrophilic AFM tip. These features are much smaller than the 20−50 Å root mean square gold surface roughness. Identical lines resulted from patterning speeds varying from 10 to 320 μm/s.

3 orders of magnitude faster than traditional AFM-based nanografting, more that 2 orders of magnitude faster than DPN, and more that an order of magnitude faster than STM-based nanografting).

The force the AFM tip applies to the substrate is equal to the sum of the force due to the flexing of the cantilever (controlled by the AFM feedback—1.0 nN in these experiments) and the attractive forces between the cantilever and the substrate (referred to as the "meniscus force" in this paper). The meniscus force is equal to the surface tension of the liquid film (about 70 mN/m for water and most aqueous solutions) times the length of the contact line around the AFM tip (Figure 1). The length of the contact line depends on the thickness of the film and can be micrometers (as judged by the size of the drop of fluid on the AFM tip, as observed by optical microscopy, and the known geometry of the AFM tip[13]) yielding an AFM tip contact force of up to a micronewton—an enormous

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(12) The AFM used was the "CP" by Thermomicroscopes.
(13) Thermomicroscopes' Sharpened Microlever. The tip is 3 μm long and shaped like a pyramid. The cantilever is 180 μm long and has a spring constant of 0.05 N/m.

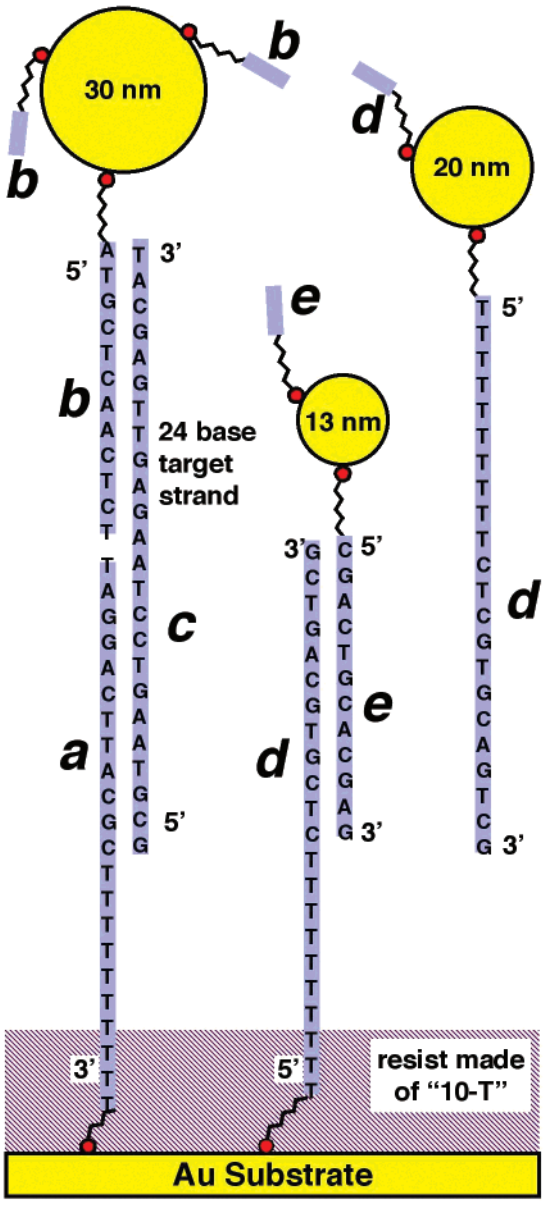

Figure 3. Hybridizing DNA-derivatized gold nanoparticles. Shown are the three-component and two-component systems used to identify surface-bound nanostructures of DNA. The "10-T tethered recognition strands" are patterned through the resist on the gold surface. The resist (a layer of thiol-modified 10-T) also prevents the nonspecific bonding of nanoparticles to the gold surface. Nanoparticles can be directly hybridized (via hybridization of sequences "d" and "e") or via the 24-base target strand (via hybridization of sequences of "a", "b", and "c"). Surface-bound nanoparticles can also be bound to other nanoparticles: the DNA sequence attached to the 20 nm nanoparticle is complementary to that attached to the 13 nm nanoparticle. Nanoparticles are derivatized with *many* strands of DNA of the same sequence,[21] although only a few are indicated in this representation.

force for an AFM tip. As this force greatly exceeds the 1.0 nN force set by the flexing of the cantilever, the "cantilever feedback force" can be neglected. A micronewton attractive force between the AFM tip[13] and the substrate would result in a downward deflection of the AFM tip of about 6°, making an exact measurement of the contact force impossible with an AFM force curve. However, observations of the cantilever's deflection with an optical microscope put the attractive force between 200 nN and 2 μN. The micronewton contact force between the silicon nitride

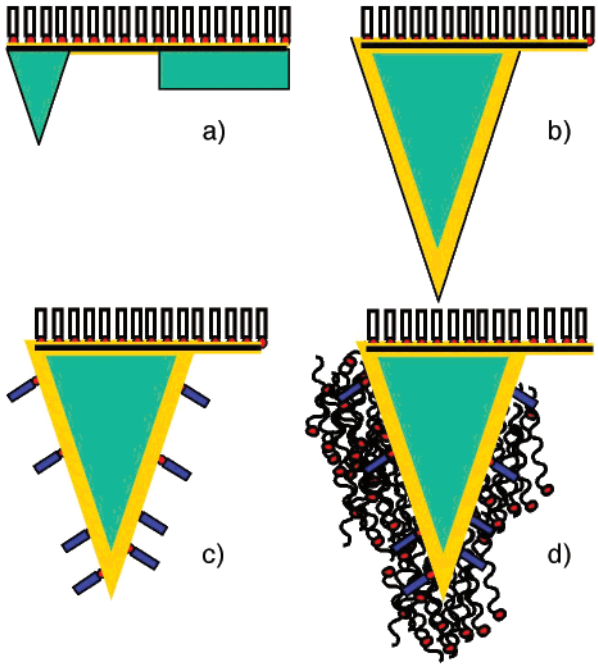

Figure 4. AFM coating protocol: (a) a passivating monolayer of ODT is added to the top, reflecting surface of the AFM cantilever, making it hydrophobic; (b) the AFM tip is gold coated with a titanium binder; (c) thiol-modified 10-T is adsorbed on the tip, making the under side hydrophilic; (d) subsequent immersion in DNA recognition strand solution results in adsorption on the tip only.

AFM tip and the gold surface produces gigapascal pressures over the contact area of the AFM tip (the radius of the contact area can vary from about 10 to about 100 nm)—well past the yielding strength of the gold substrate. As a result, not only is the resist removed but also a flattened line on the gold surface is left in the path of the AFM tip. Figure 2 is an AFM topograph of a resist-covered gold surface immediately after the MFN patterning of vertical lines. Beneath the topograph is a cross-sectional average of the image above. Indentations from a sharp tip are still less than a nanometer deep—a small dent in a gold surface having more than 2 nm root mean square surface roughness (the 2 nm root mean square surface roughness is not apparent because of the line averaging). A sharp tip produces indentations that are about 10 nm wide, but a tip with a flattened point will produce a wider mark, and less gold indentation. Although we have conducted MFN on a particularly hydrophilic surface (DNA), the process should work as long as a liquid is used that wets both the AFM tip and the sample. The strength of the attractive force, and thus the extent of gold surface flattening, can be controlled by the depth of the drop of patterning solution.

Detection of DNA Nanostructures

The detection of nanostructures of surface-bound DNA presents a challenge in and of itself. Traditionally, surface-bound DNA has been detected through hybridization to its fluorescent-labeled complement before imaging with fluorescence microscopy, limiting the resolution of the detection mechanism to the wavelength of light used. The use of DNA-derivatized magnetic microbeads[14] to detect

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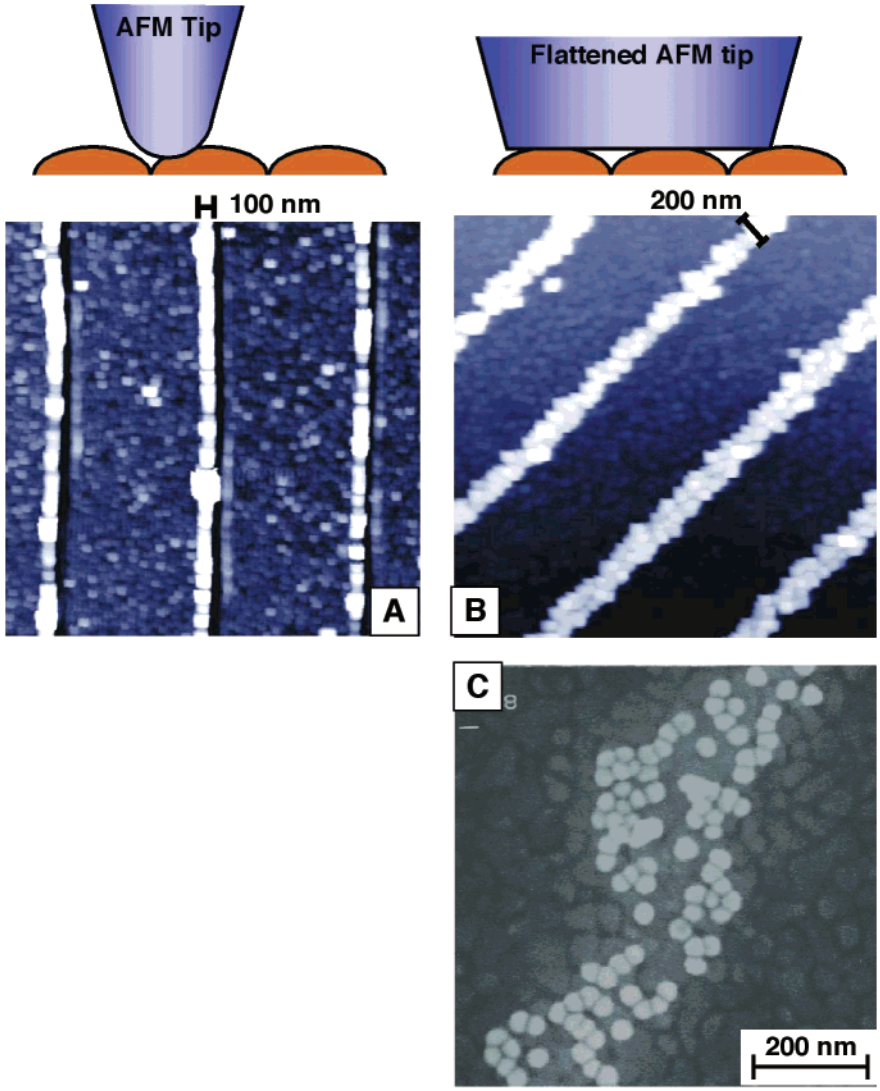

Figure 5. 30 nm nanoparticles hybridized to a DNA MFN-patterned gold surface. (A) A sharp tip produces a thin line (AFM topograph). If the patterned DNA line is smaller than the nanoparticle, the nanoparticles are forced into a straight line. Lines appear wider than they actually are due the convolution of the tip of the AFM, which has a diameter of about 60 nm. (B) A flattened tip produces a wide line but does not nanograft to the lower regions (AFM topograph). (C) SEM close up of sample shown in "B" shows nanoparticle distribution. MFN-patterning of DNA produced near-identical marks for writing speeds of 10−320 μm/s.

surface-bound DNA also has the spatial precision of approximately a micrometer. Recently, solution-born DNA fragments have been detected through the use of DNA-derivatized gold nanoparticles:[15] the target sequence to be detected (sequence "c", see Figure 3) attaches DNA-derivatized nanoparticles to a DNA-derivatized glass surface via a "three-component nanoparticle hybridization system". One can use this same strategy to detect the presence of patterned DNA nanostructures on a gold surface: by hybridizing DNA-derivatized gold nanoparticles to the patterned DNA. As a single surface-bound nanoparticle can be detected using AFM or SEM, this technique promises the ability to detect a single strand of patterned DNA with spatial resolution equal to the radius of a gold nanoparticle (presently as small as 7.5 Å).[16]

DNA-derivatized nanoparticles were made according to the literature.[17] Nanoparticles were hybridized to the patterned surface by immersing the surface for an hour in a buffer solution (0.3 M NaCl, 10 mM phosphate, pH = 7) containing nanomoles per liter concentrations of the appropriate nanoparticles. For the three-component system, the 24-base target strand is hybridized to the surface-bound DNA prior to nanoparticle application, by immersing the patterned sample in 10 μM DNA in buffer solution (1 M NaCl, 10 mM phosphate, pH = 7) for an hour.

Detection of DNA on a gold surface presents the added difficulty of eliminating the nonspecific binding of nanoparticles. Unlike a glass surface, the DNA-derivatized nanoparticles are strongly attracted to a gold surface because of the image charge in the conductor, and they bind irreversibly. We have found that this nonspecific binding of nanoparticles can be eliminated by adsorbing a monolayer of thiol-modified DNA strands (consisting of 10 thymine or "10-T") on the gold surface.[18] The 10-T strands pack tightly on the gold surface, providing a strong

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negative surface charge, which repels the negatively charged DNA nanoparticles. A 10-T resist is established on the gold surface by immersing the surface in a (10 μM DNA/1 M $MgCl_2$) solution of thiol-modified 10-T DNA for an hour or longer. The solution can be left to equilibrate with the atmosphere, as it will concentrate, but not dry.

MFN Patterning of DNA

Pure DNA is an immobile ionic polymer, but it increasingly adsorbs water of hydration with increasing relative humidity and we have found that pure DNA rather abruptly "melts" at a relative humidity above 90%. This "melting humidity" can be lowered with the addition of $MgCl_2$ salt. The molar ratio of salt to DNA bases (salt to base ratio, or SBR) and the relative humidity are important in determining the fluidity of the bulk DNA/$MgCl_2$ mixture. We have found that a DNA/$MgCl_2$ mixture with an SBR of 27 (for our 22-base thiol-modified recognition strands) remained manageably fluid when in equilibrium with our laboratory atmosphere (35%−50% relative humidity). This DNA recognition strand/salt mixture was adsorbed onto a hydrophilic AFM tip and deposited directly on the resist-coated gold surface.

It is important that the AFM tip is hydrophilic, but the back of the cantilever is hydrophobic (to prevent adsorption of DNA on the reflective surface). The gold-coated tips where immersed in 1 mM octadecanethiol/acetonitrile for an hour to establish a hydrophobic reflective surface on the back of the cantilevers (see Figure 4). The other side of the cantilever (the "tip side") was then gold coated (5−10 nm of gold was evaporated after a 5 nm titanium binder), and the tips were immersed in 10 μM thiol-modified 10-T/1 M $MgCl_2$ for more than an hour. The adsorption of the DNA on the gold-coated tip made the tip very hydrophilic. Subsequently, $MgCl_2$ solution of thiol-modified DNA recognition strand adheres to the AFM tip, but not to the back of the cantilever.

Crucial to MFN is the passivation of the substrate from unwanted binding of patterning molecules: establishing a resistive film. The 10-T resist does not protect the gold surface from subsequent adsorption of thiol-modified DNA during bulk DNA deposition (10 μM DNA/1 M $MgCl_2$), as it does not form a well-packed, self-assembled monolayer.[19] However, under a film of the somewhat viscous DNA/$MgCl_2$ solution at equilibrium hydration described above, the thiol-modified DNA recognition strand is observed to *not* penetrate the resist. However, after the 10-T resist is removed from the surface by the AFM tip, recognition strands chemisorb to the patterned gold surface. The substrate is rinsed in Nanopure water immediately after patterning. After the MFN application of one sequence of DNA, the patterned surface can be passivated to additional DNA applications by again applying the 10-T resist. We have found that if the 10-T is not applied after the patterning of each DNA sequence, patterned areas are not protected from subsequently applied DNA.

Results of the hybridization of the 30 nm nanoparticles via the three-component system are shown in AFM topographs and SEMs (Figure 5). Patterning with a sharp tip (Figure 5A) results in a continuous, fine line indicated by the single-file row of 30 nm nanoparticles. The nanoparticles appear much larger in the AFM micrographs as a result of significant tip convolution. As the nanoparticles are staggered by less than 15 nm, it is reasonable to assume that the width of the patterned DNA line is as thin as 15 nm, consistent with other reports of nanografting. A wider line results from a suspected flattened tip, where the surface of the AFM tip would only flatten the high parts of the polycrystalline gold surface (Figure 5B, Figure 5C). Observation of the SEM indicates a nanoparticle surface coverage that is slightly greater than 50% in the patterned areas, and the unintentional binding of four and two nanoparticles (in panels A and B of Figure 5, respectively) represents 0.04% and 0.02%, respectively. This corresponds to a selectivity of about 2000:1.

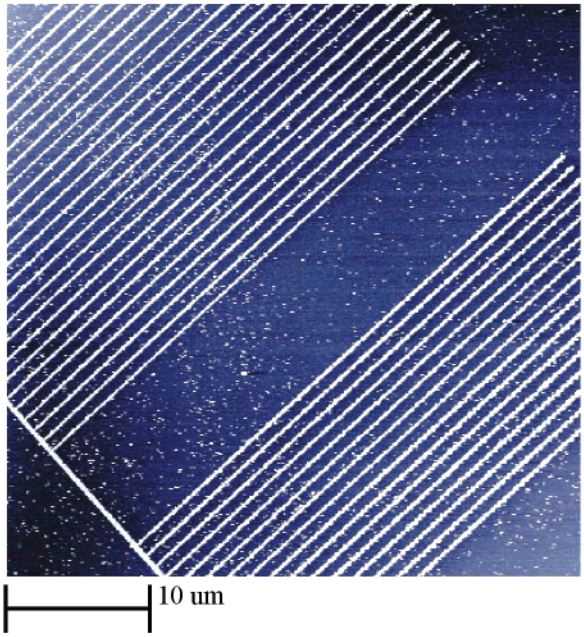

Figure 6. Line speed independence. AFM topography of same sample as panels B and C of Figure 5. 30 nm nanoparticles hybridized to MFN-generated DNA lines. These lines were patterned with speeds that ranged from 20 to 160 μm/s.

The high-speed capability and reproducibility of MFN pattering are well demonstrated in Figure 6 which is a wide view, AFM micrograph of the substrate depicted in Figure 5B. The lines shown were patterned at tip speeds ranging from 20 to 160 μm/s. We have successfully patterned at speeds from 10 to 320 μm/s. Higher speed patterning has not yet been attempted.

Surface Initiated Orthogonal Self-Assembly

By patterning lines of different DNA sequences, we have been able to independently direct the assembly of different nanostructures on the same gold surface. Parallel lines of recognition strands "a" (see Figure 3) were MFN patterned next to lines of recognition strand "d", with less than 500 nm spacing between the lines. Between patterning the two different sequences, the 10-T resist was reapplied in order to prevent the lines that were patterned first from becoming contaminated with the sequence that was patterned second. After patterning the two different recognition strands, 13 nm gold nanoparticles, derivatized with DNA sequence "e", were hybridized to the patterned surface (Figure 7A shows an AFM topograph. The graph at the top of the image is the average height in the white box shown in the image.), followed by the hybridization of 30 nm nanoparticles (Figure 7B). The two different kinds of nanoparticles adsorbed only to the patterned lines of the corresponding complementary recognition strands, as shown. Twenty nanometer nanoparticles were then hybridized to the line of 13 nm nanoparticles (Figure 7C) as a demonstration of how orthogonal self-assembly can

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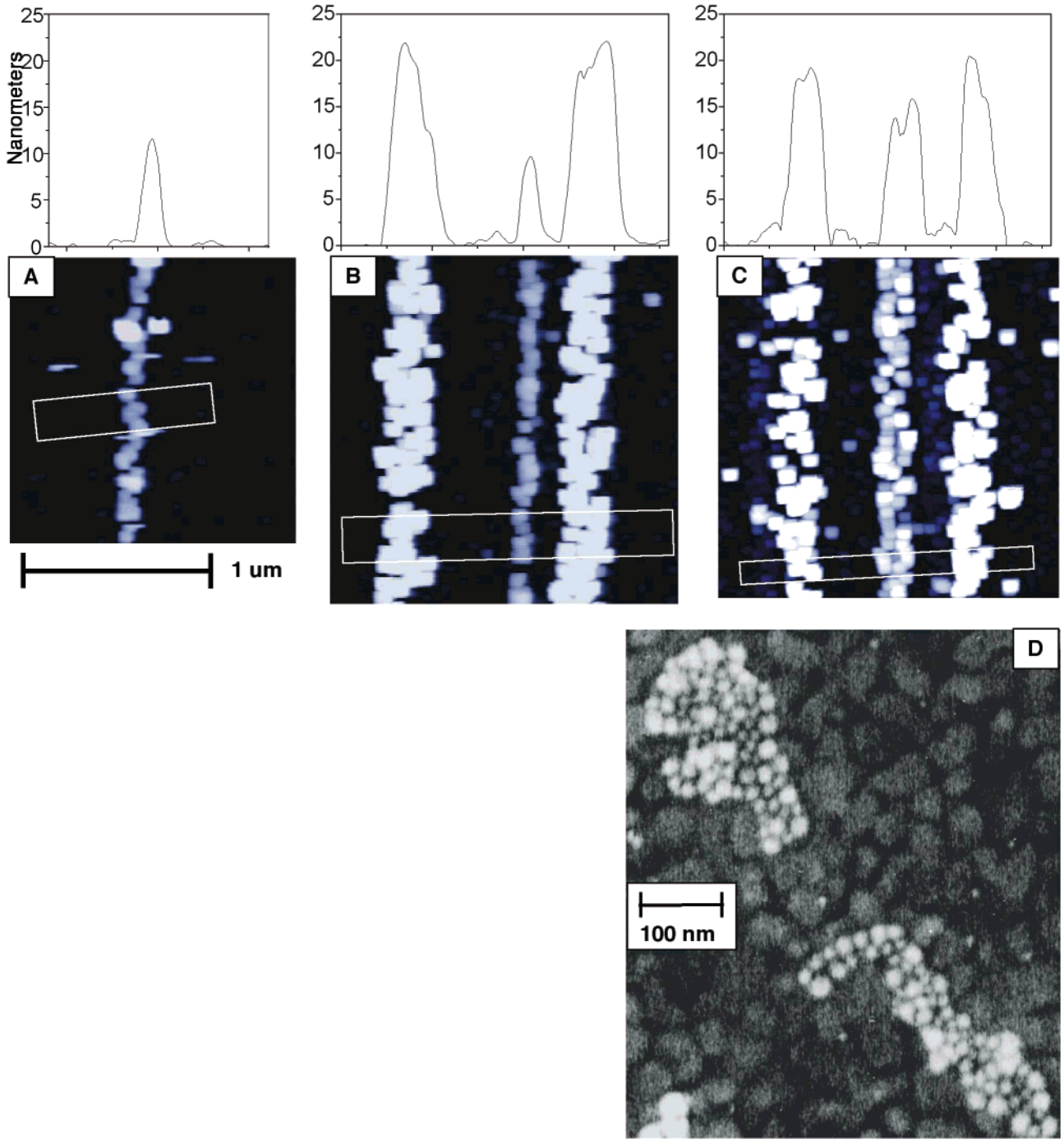

Figure 7. Surface initiated orthogonal self-assembly. Separate lines of recognition strands "a" and "d" were MFN patterned on a gold surface. (A) 13 nm nanoparticles were first assembled, followed by (B) the 30 nm nanoparticles—each to the appropriate line containing the complementary sequence. (C) Last, 20 nm nanoparticles were hybridized to the 13 nm nanoparticles. Above each AFM topograph is the average height in the box drawn on each image. (D) An SEM image allows one to distinguish the 13 nm nanoparticles from the 20 nm nanoparticles on the same (diagonal, discontinuous) line and the 30 nm nanoparticles (lower left).

continue with the application of additional layers. An SEM image (Figure 7D) allows one to clearly distinguish the 20 and 13 nm nanoparticles on the same (discontinuous, diagonal) line and the 30 nm nanoparticles (lower left).

Conclusions

The speed and versatility of MFN for patterning solution-born molecules on a substrate with nanometer precision gives it potential industrial viability in fields ranging from biosensing and biotechnology to catalysis, nanotechnology, and the computer industry. Moreover, we have shown that by greatly increasing the normal force on the AFM tip during nanografting, the gold surface is slightly flattened, allowing the process to be conducted on a polycrystalline surface at speeds more than 3 orders of magnitude faster than when done at lower force. This high force nanografting (HFN) also has other promising variations besides MFN. High normal forces can be achieved in other ways—such as through the traditional flexing of the cantilevers, or through an external force (for instance that of a magnetic field[20]). Patterning molecules could also be delivered to the resist-covered

substrate prior to patterning by a variety of methods such as solution deposition or some form of vapor deposition technique. In particular, the ability to pattern DNA holds promise, not just toward improved genetic detection but as a means to direct the three-dimensional assembly of nanostructures (such as future components of molecular electronics)—a first step in the direction of which we have demonstrated here.

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Addendum. This work was done in the laboratories of the Mirkin Group at Northwestern University while Peter Schwartz was a postdoctoral associate working with us. While we all contributed to many of the ideas and experiments presented in the manuscript, to the best of our knowledge much of the work has yet to be reproduced by Schwartz or by us. We believe that some of the conclusions may be erroneous, and we intend to correct them in a future manuscript that credits all of those involved with the work. Until the data have been reproduced and the proper control experiments have been done, none of us feels comfortable including our names as coauthors.

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