Atomic force microscopy reveals two phases in single stranded DNA self-assembled monolayers

Priscila Kosaka*, Sheila González*, Carmen Martínez, Alfonso Cebollada, Alvaro San Paulo, Montserrat Calleja and Javier Tamayo*

Bionanomechanics lab, Institute of Microelectronics of Madrid, CSIC, Isaac Newton 8 (PTM), Tres Cantos, 28760 Madrid, Spain.

* Address correspondence to jtamayo@imm.cnm.csic.es

We have investigated the structure of single-stranded (ss) DNA self-assembled monolayers (SAMs) on gold by combining peak force tapping, Kelvin probe and phase contrast atomic force microscopy (AFM) techniques. The adhesion, surface potential and phase shift signals show heterogeneities in the DNA film structure at two levels: microscale and nanoscale; which cannot be clearly discerned in the topography. Firstly, there is multilayer aggregation covering less than 5% of the surface. The DNA multilayers seem to be ordered phases and their existence suggests that DNA end-to-end interaction can play a role in the self-assembly process. Secondly, we find the formation of two phases in the DNA monolayer, which differ both in surface energy and surface potential. We relate the two domains to differences in the packing density and in the ssDNA conformation. The discovered heterogeneities in ssDNA SAMs provide a new scenario in our vision of these relevant films that have direct consequences on their biological, chemical and physical properties.

1. INTRODUCTION

Thiolated self-assembled monolayers are spontaneously formed on gold by the adsorption of molecules with one thiol end that strongly binds the gold surface, and other end that has weak affinity to the surface and provides the surface functionality\textsuperscript{1,2}. The functional and thiol groups are spaced by an alkane chain. SAMs generate well-defined organic surfaces with tunable chemical, optical, electrical and tribological functionalities\textsuperscript{3}. Despite the extensive studies that SAMs have attracted, the dynamics and kinetics of the assembly remain incompletely understood. For instance, decanethiol SAMs used as a model system exhibits the formation of six different phases that strongly depends on the coverage\textsuperscript{4,5}. In a simplistic picture, the SAMs growth initially involves physisorbed and mobile thiols, and chemisorbed thiolates lying flat on the surface with noncrystalline geometry. This domain remains until \~90% of the final coverage is reached in few minutes. Then a domain transition to a highly packed ordered monolayer with the molecules upright-oriented occurs driven by the attractive lateral interactions (van der Waals, hydrogen bonding) within the geometric constraints imposed by the previous structure of the Au-S binding sites\textsuperscript{1,5-6}. The density of this domain is limited by the steric crowding interactions. The domain transition from the disordered to the ordered domain constitutes a significant kinetic bottleneck for long-chains that can last from hours to days.

Self-assembled monolayers of single stranded DNA on gold are a relevant and peculiar case of thiolate self-assembled monolayer\textsuperscript{7,9}. In this case, short ssDNA probes, 12-30 bases
long, are derivatized with a thiol linker at one of the DNA ends and diluted in a buffer solution. A DNA SAM in which the ssDNA molecules are randomly coiled and anchored to the gold via the thiol group is obtained after long incubation (24-48 h). DNA SAMs are used in a variety of biotechnology and nanotechnology applications. Thus, there exist a variety of nucleic acid biosensors such as electrochemical\(^{10}\), nanomechanical\(^{11-16}\) and optical\(^{7-19}\) biosensors based on ssDNA SAMs. In addition, Au nanoparticles can routinely be synthesized using various methods, and can be functionalized with ssDNA SAMs to develop ultrasensitive DNA sensors\(^{20-22}\). In addition, DNA functionalized Au nanoparticles as well as DNA SAMs on Au surfaces have been used to build a variety of nanoscale structures with potential applications in biomimetic systems and novel electronic and photonic devices\(^{23-24}\). Strikingly, DNA SAMs also exhibit outstanding physical properties such as spin-selectivity in the transmission of electrons through self-assembled monolayers of double-stranded DNA on gold\(^{25}\).

The peculiarity of DNA SAMs comes from i) the extraordinary size of the DNA molecule, \(\sim 10^3\) atoms in comparison with archetypal alkanethiols that have tens of atoms and ii) the high negative charge of the phosphate backbone. These features induce large steric and electrostatic repulsion between the chains that should hamper the self-assembly process. X-ray photoelectron (XPS), Fourier transform infrared (FTIR) and X-ray absorption fine structure (NEXAFS) spectroscopy have shed light on the surface density and chemical structure of these monolayers\(^{7,9}\). Briefly, film structure evolves from the DNA molecules initially lying nearly flat on the surface anchored by multiple sites, to randomly coiled ssDNA molecules anchored via the thiol group. Depending on the buffer ionic strength and DNA length, a highly packed monolayer is achieved after 24-48 hours with a surface coverage of 2-8x10\(^{15}\) molecules/cm\(^2\). Surprisingly, the highest coverage corresponds to an average spacing between individual ssDNA probes of 1-1.8 nm that is close to the radius of the DNA double helix\(^8\). This high packing is unexpected due to the strong electrostatic and steric repulsive interactions. This finding raises the question about what attractive interaction drives the ssDNA monolayer assembly. It seems that nucleobase stacking that largely determine the structure and interactions of DNA including DNA hybridization can assist the assembly process and provide local ordering in the film\(^9\).

Most of the information about DNA SAMs represents an average picture that ignores the surface heterogeneities. It is widely assumed that DNA SAMs form homogeneous monolayers. Recently, this picture has been broken by a study that combines electrochemistry and fluorescence that shows that ssDNA SAMs passivated with mercaptohexanol exhibit significant surface heterogeneity\(^{26}\). However, the limited spatial resolution (\(\sim 1\) µm) and the distance-dependent quenching of the fluorescence unable to precisely dissect the structural surface heterogeneities.

In this work, we use a powerful combination of atomic force microscopy techniques\(^{27-28}\), peak force tapping (PFT)\(^{29}\), phase imaging and Kelvin probe (KP)\(^{30}\), to answer the question whether ssDNA SAMs are heterogeneous, and if so, to tackle the structural origin of the heterogeneity.

2. MATERIALS AND METHODS

2.1. DNA SAMs
A layer of gold with a thickness that ranges from 20 to 50 nm was deposited on silicon substrates by Knudsen fusion after a 2-5 nm thick adhesion layer of chromium, at a small deposition rate in order to decrease surface roughness (0.01-0.02 nm/s). The deposited gold films are polycrystalline with a grain size that increases from 20-30 nm for the 20 nm thick Au
layers to 80-90 nm for the 50 nm thick Au layer. We did not found systematic differences in the DNA SAM morphologies as a function of the Au layer thickness. Since the technological interest of thin gold layers, the results in the manuscript are obtained with 20 nm thick Au layers. Prior to immobilization, gold surfaces were cleaned with piranha solution (3 H$_2$SO$_4$: 1 H$_2$O$_2$) (caution: piranha solutions are extremely corrosive, reactive, and potentially explosive) for 10 minutes, rinsed three times with deionized water (18.2 MΩ·cm) and finally dried under a stream of dry nitrogen (N$_2$). The freshly cleaned substrates were immediately incubated with 5 μM thiol-modified ssDNA probe in PBS buffer (pH 7) with 1M NaCl, for 48 hours at room temperature to ensure the formation of a highly packed monolayer. After incubation, the samples were extensively rinsed with PBS buffer with 1M NaCl and three times with DNase/RNase free water to remove unbounded material and finally dried with N$_2$. In this study, we use three different DNA sequences of 25, 19 and 16 bases obtaining similar results: 5’HS–C$_6$H$_{12}$–ACT GCA ACC AGT TTC CTC TTG GGT G 3’, 5’HS–C$_6$H$_{12}$–GTC GGA CTC AAG CTA TCA C 3’ and 5’HS–C$_6$H$_{12}$–CTA CTTTT TTT TCT G 3’. The results shown in the images were obtained with the 19mer ssDNA.

2.2. AFM

The AFM measurements were performed in air at 24-26 ºC and 20-40% relative humidity with a Bruker Multimode AFM with a Nanoscope V controller and with highly doped silicon probes with 2 nm and 0.4 N/m nominal tip radius and spring constant. Peak Force Tapping$^\text{TM}$ and Kelvin Probe modes were applied to study the structure of the ssDNA SAMs. The methodology involved in these modes is sketched in Fig. 1. In PFT-AFM, the tip-sample distance is periodically modulated at 2 kHz in order to acquire force-distance curves at high speed during the sample scanning (Fig. 1(a))$^{29}$. The modulation frequency is well-below the cantilever resonance frequency, thus the force-distance curves can be easily interpreted by static analysis. Real-time fitting of the force-distance curves provides adhesion, sample deformation and a variety of sample elasticity parameters by applying different contact mechanics models. The maximum tip-sample repulsive force established during the tip-sample contact provides the feedback signal to obtain the topography of the surface. In this work, we have used the adhesion channel, as information on the mechanical properties requires of high forces that compromise the spatial resolution.

In KP-AFM, each line is scanned twice (Fig. 1(b))$^{30}$. During the first pass, the cantilever is mechanically excited at near its resonance frequency, and the amplitude is kept constant by a feedback system to obtain the topography. Simultaneously, the phase shift between the cantilever oscillation and the driving signal is recorded to obtain compositional contrast of the surface based on difference in inelastic interactions between the tip and the sample$^{31-32}$. In this operation mode, the tip-sample interactions are largely minimized leading to higher spatial resolution in comparison to PFT-AFM. However, the phase contrast cannot be simply interpreted in terms of adhesion and mechanical properties of the sample. In the second pass, the cantilever tracks the previously recorded height profile at a set lift height, 15-50 nm, from the sample surface to detect the electric surface potential. During this second pass the cantilever is no longer excited mechanically but electrically by applying to the tip a voltage containing a DC component and a AC component at the resonance frequency ($\omega_0$),

$$V_{tip} = V_{DC} + V_{AC} \cos(\omega_0 t)$$  \hspace{1cm} (1)

The resulting capacitive force between the tip and the surface is,

$$F_{cap} \approx \frac{1}{2} \frac{\partial C}{\partial z} \left( V_{tip} - \phi(x) \right)$$  \hspace{1cm} (2)

3
where \( C(z) \) is the capacitance, \( z \) is the separation and \( \Phi(x) \) is the difference in surface potential between the tip and the sample. The force component at the resonance frequency is,

\[
F_{\text{cap, } \omega_0} \approx \frac{\partial C}{\partial z} (V_{DC} - \Phi(x)) V_{AC}
\]  

A feedback is applied that changes the DC tip potential, until the cantilever vibration at resonance vanishes, so \( V_{DC}(x) \) becomes equal to \( \Phi(x) \). Thus the image obtained by recording the DC tip voltage reflects spatial variations of the surface potential along the sample surface.

### 3. RESULTS

Figure 2(a) shows the PFT-AFM topography of a 20x20 \( \mu m^2 \) region of a thin gold-film functionalized with the 19 mer ssDNA probe. The image shows a uniform ssDNA monolayer with physisorbed multilayer islands covering 18\% of the image. Generally, multilayers are randomly dispersed covering less than 5\% of the sample. The islands have a thickness that is a multiple of 4.5±0.5 nm. Single stranded DNA in solution behaves as a flexible polymer that adopts random coiled conformation. In this case, the DNA length (including the thiol linker) and persistence length are of about 8.2 nm and 1 nm, respectively\(^{23}\). The mean end-to-end distance using the worm-like chain model is of about 4 nm that is similar to the thickness of the physisorbed monolayers\(^{14}\). We conclude that the DNA is in an upright and coiled conformation in the physisorbed islands. These kinds of structures, observed here for first time, are unexpected due to the strong electrostatic repulsion between the negatively charged DNA chains. The islands exhibit well-defined edges that form angles of 120±15 degrees, suggesting that the DNA molecules form an ordered domain with hexagonal packing. We will discuss later the mechanism that may lead to these structures. Figure 2(b) shows the adhesion image simultaneously acquired with the topography. Independently of the number of layers, the physisorbed islands show uniform adhesion, which is the lowest in the image. Surprisingly, the adhesion channel reveals that the ssDNA monolayer exhibits two phases, one that provides 1.7 times higher adhesion than the islands, and other that provides almost three times higher adhesion. The histograms of height and adhesion values are plotted in Figs. 2(c) and 2(d), respectively. The height histogram exhibits four Gaussian peaks corresponding to the monolayer and the islands of one, two and three ssDNA layers. The adhesion indicates three levels of adhesion, one for the physisorbed islands, and two for the monolayer as described above. We will hereinafter focus on to elucidate the origin of the phase separation in the ssDNA monolayer.

Figure 3(a) shows the topography of a 5x5 \( \mu m^2 \) region of another representative sample. The topography shows the typical grain structure of polycrystalline gold. The adhesion channel, Fig. 3(b) reveals two phases in the monolayer that are not perceptible in the topography image. The histogram of the adhesion values, Fig. 3(c), fits extremely well with a bimodal Gaussian distribution. The ratio between the adhesion positions of the two peaks is 1.3. The ratio between the adhesion values of the domains ranges from 1.2 to 1.7 between different samples. To get further insight on the structural origin of the two domains in the DNA SAM, we imaged a 500x500 nm\(^2 \) region around the domain boundary marked in Fig. 3(b) at our highest resolution (Fig. 4). The topography shows the typical grain structure of the polycrystalline gold. The grain size ranges from 50 to 80 nm and many of the grains exhibit small height depressions near the grain center that are typical structural defects in SAMs\(^2 \) (Fig. 4(a)). These defects will be used below to estimate the monolayer thickness. The two phases in the DNA SAM cannot be clearly distinguished in the topography image. However, the adhesion
image (Fig. 4(b)) clearly reveals the two domains. Interestingly, in the domain with lower mean adhesion, the values of adhesion are nearly uniform, although the topography exhibits significant roughness due to the gold grain faceting lined up with the (111) crystal directions. On the contrary, we observe nanoscale adhesion heterogeneities in the domain with higher mean adhesion. The differences between the two domains can be more clearly determined in the cross-section across the domain boundary shown in Fig. 4(c). Firstly, we find that the regions between grains are deeper in the domain of higher mean adhesion. In addition, the grain edges are more sharply defined. Secondly, in the domain of higher mean adhesion, the intergrain regions provide significantly higher local adhesion than the top, whereas this adhesion contrast is not found in the domain of lower mean adhesion. We have found that bare gold surfaces provide significantly higher adhesion than ssDNA covered surfaces, which implies the higher the DNA density, the lower the adhesion. Thus, our results suggest that in the domain of lower mean adhesion, the gold grains are uniformly covered by a highly packed ssDNA monolayer, whereas in the domain of higher mean adhesion most of the DNA molecules assemble on the top surface of the grains with a similar conformation, although less packed. In the regions between grains, the density of DNA molecules would be significantly smaller and probably in a lying-down conformation.

In order to confirm that the regions of lower adhesion correspond with regions of higher packing of the ssDNA SAM, we applied Kelvin-probe AFM. In this imaging mode, each scan line is scanned twice, the first generates the topography in the tapping mode, the second tracks the obtained height profile at a higher separation, from 15 to 50 nm, to record the surface potential. Previous works of Kelvin probe microscopy on DNA SAMs demonstrate a correlation between the surface potential and the density of the monolayer. Figure 5 shows the topography (a) and surface potential (b) images of a 5x5 µm² region of a thin gold layer functionalized with the ssDNA SAM. As described above, the topography shows the typical grain morphology of polycrystalline gold with no evidences of phase segregation. However, the surface potential image shows two domains similar to those revealed by adhesion in the PFT mode. The region of more negative potential is attributed to a higher DNA density due to the negatively charged DNA backbone. We use the stiff cantilevers required for optimal KP-AFM, for PFT-AFM imaging of the same region. Despite the higher force noise, the force sensitivity was enough to check the adhesion difference between the regions with different surface potential (data not shown here). The measurements demonstrate that the regions of more negative surface potential (higher ssDNA density) correspond to regions of lower adhesion, as we hypothesized above.

Changes in the surface potential depend on the molecular dipoles, surface coverage, and conformation of the adsorbed molecules. By modeling each DNA molecule in the monolayer as an effective dipole with moment µ tilted relative to the surface normal by θ, the surface potential variation is given by,

$$\Delta V = \frac{N\mu \cos \theta}{\varepsilon_r \varepsilon_0}$$

(4)

Where N is the molecular density, $\varepsilon_r$ is the effective dielectric constant of the DNA film, and $\varepsilon_0$ is the vacuum permittivity. The effective dipole moment arises from the electron transfer from the gold to the sulfur, and from the highly negatively charged DNA backbone and the counterion shielding. Recent studies indicate that the last contribution dominates in dense DNA films and the change of surface potential approximately is proportional to the molecular density (N), with little contribution from the conformation ($\theta$). By adopting this assumption, we estimate a difference in DNA density of 15-20% between these two domains.
A high resolution image of the boundary region of 300x300 nm² marked on fig. 5(b) is shown in figure 6. The topography shows the gold grains with depression defects near the grain center (Fig. 6(a)). We notice that the tapping mode provides higher resolution than the peak-force mode. This is probably related to the inherently smaller tip-sample interactions achieved in the dynamic mode operation. The depression defects in highly packed monolayers can be related to boundaries between molecular domains with different titling orientations, but identical packing density. Thus, these depressions are defective regions in the SAM that enable to estimate a lower limit to the monolayer thickness. By analyzing the depth of these defects in several images, we estimate that the monolayer thickness in the domain of more negative potential is of about 4.2±0.5 nm, similar to the monolayer thickness of the physisorbed islands, whereas for the domain of less negative surface potential, the estimated thickness is of about 3.2±0.4 nm. As shown above, Kelvin probe microscopy can provide quantitative information about the DNA SAM conformation and density, but its spatial resolution is limited due the long-range nature of the electrostatic interactions that involves a significant part of the microscopic tip and cantilever. In our case, from the surface potential change at the domain boundary (Fig. 6(b)), we estimate an effective diameter of the area of the sample that interacts with the AFM tip of 0.6 µm. To image the nanoscale heterogeneities, we monitored the phase lag between the cantilever oscillation and the driving signal during the first scan line in the KP-AFM (Fig. 6(c)). We find that phase shift image correlates to the surface potential image, but it provides higher spatial resolution due to the lower mean separation between the tip and the surface. Similarly to the adhesion images, the phase image exhibits nearly uniform phase in the region of more negative surface potential, whereas shows nanoscale heterogeneities in the region of less negative surface potential. We plot the cross-sections of the topography and phase shift along two paths in each monolayer domain (Fig. 6(c)). In the region of less negative surface potential, the phase shift is significantly higher in the region between grains, and grain edges, whereas it reaches a value at the grain top region that is slightly higher than that in the domain of more negative surface potential. This behavior is identical to that of the adhesion shown above, and reflects that the energy dissipation that dictates the phase shift contrast is related to adhesion differences, probably as a consequence of the adhesion hysteresis.

4. DISCUSSION

It has been largely assumed that the self-assembly of thiolated DNA probes leads to uniform monolayers. Recently, a study based on electrochemical in-situ fluorescence microscopy modified this picture. DNA SAMs backfilled with MCH exhibited heterogeneous surface coverage with hot spots of intense fluorescence due to aggregates of physisorbed structures. The structural origin of these heterogeneities could not be clearly elucidated due to the limitations of the fluorescence technique. Firstly, fluorescence has a limited spatial resolution (∼1 µm). Secondly, the packing density and DNA conformation are entangled in the fluorescence signal due to the distance-dependent quenching of the fluorescence by the metal. In addition, the ‘native’ structure of the DNA monolayer can be modified by the fluorescent tag. Here, we use two powerful AFM techniques to study ssDNA SAMs, a new emerging mode referred to as peak force tapping, and Kelvin probe that has recently demonstrated the capability to characterize layers of charged biomolecules. Our results demonstrate that the highly packed DNA monolayers on gold obtained by self-assembly are not uniform. We observe two phenomena: multilayer aggregation and two-phase separation in the monolayer. These findings illustrate the complexity of the growth of DNA SAMs that is governed by an unprecedented variety of interactions such as steric, conformational entropy, electrostatic, base-stacking and hydrophobic interactions.
DNA multilayers physisorbed on the monolayer exhibited well-defined edges reminiscent of crystalline nature. We believe that these multilayers correspond with the fluorescence hot spots previously reported\textsuperscript{38-39}. We speculate that the end-to-end DNA stacking interactions, a recently discovered form of DNA self-assembly, can contribute to the stabilization of these structures, which are unexpected when electrostatic interactions are accounted for\textsuperscript{38-39}. However, these attractive interactions have been only detected between duplex DNA. We have estimated the surface density of our monolayers by radiolabelling, X-ray photoelectron spectroscopy and microbalance based on bulk resonators\textsuperscript{11}. The assays indicate an average density of 3.2±0.2 × 10\textsuperscript{13} mol/cm\textsuperscript{2} that implies that DNA molecules in the monolayer are separated by = 1.3 nm that is near the radius of the DNA duplex. We speculate that this high packing can act as a template for the end-to-end stacking of ssDNA molecules giving rise to the crystalline islands. Further investigation will be needed to determine the mechanisms that originate these layers.

Our second finding is also remarkably intriguing: the DNA monolayer exhibits two domains that differ in surface energy and surface potential. Despite the high resolution of AFM, the domain separation in the monolayer cannot be clearly discerned in the topography. This is due to the small difference in thickness of the two domains, and the grain structure of the polycrystalline gold surface that masks small height variations in the monolayer. The monolayer domain of higher DNA density is highly uniform in adhesion and tapping mode phase, i.e., the gold grains and intergrain regions are uniformly covered by DNA. The thickness of the DNA layer is approximately 4.2 nm, similar to the monolayer thickness in the physisorbed islands. This indicates that the DNA molecules adopt a coiled conformation preferentially aligned perpendicular to the substrate and are anchored to the gold via the sulfur-gold bond as previously proposed\textsuperscript{8-9}. In the less dense domain, both interdigitation between DNA segments as well as a higher tilt angle with respect to the surface normal can lead to the smaller thickness. Interestingly, we find that the less dense domain results of two nanoscale domains: one preferentially located in the top sites of the grains and other in the grain edges and regions between grains. Strikingly, the surface energy difference between these two nanoscale subdomains is approximately 30%, which suggests a significant difference in the monolayer structure. We infer that the DNA monolayer is 0.7±0.1 nm thicker at the top than at the edges and the intergrain region. This suggests that the DNA is preferentially lying down on these regions. Fig. 7(a) shows a 3D topography image obtained in tapping mode, in which the colour intensity is determined by the phase shift that in our model, is related to the DNA packing density and conformation. In the domain of higher packing (referred to as I in the figure), the grains show approximately uniform levels of DNA packing density, however, in the domain of lower packing the DNA preferentially self-assemble on the top of the grains. A schematic model is show in figure 7(b).

5. CONCLUSIONS

In contrast to what was previously assumed, our results demonstrate that the highly packed DNA monolayers on gold obtained by self-assembly are not uniform. We observe multilayer aggregation covering less than 5% of the surface and two-phase separation in the monolayer due to differences in packing density of about 10-15%. The monolayer domain with higher DNA density is highly uniform at the nanometer scale that indicates that it can represent the final state of the monolayer corresponding to a minimum in the free energy. In this domain, the DNA molecules adopt a coiled conformation preferentially aligned perpendicular to the substrate and are anchored to the gold via the sulfur-gold bond. The less dense domain exhibits nanoscale heterogeneities. DNA molecules preferentially self-assemble on the top surface of the grains, and form a lying down domain in the grain edges and
intergrain regions. This less dense domain could be a metaestable state that should finally undergo a transition to the denser and final state. However, we have not observed evolution of the phase distribution by further incubation of the sample with DNA during 12-24 hours, which indicates that the stable phase is not able to nucleate and grow at the expense of the less dense domain at room temperature or during this observation period. The significant repulsive interactions between DNA chains, mainly of steric and electrostatic nature constitute a significant kinetic bottleneck for the DNA self-assembly that can make that the self-assembly process does not reach equilibrium, even after days of incubation. We envisage that observation of the phases as a function of the temperature can help to prove the metastability of the domains. A variety of nucleic acid biosensors, nanostructures and devices are based on ssDNA SAMs. Our findings reveal nanoscale heterogeneities that can strongly influence the biological, chemical and physical properties of these films. This outlines the need of further investigation on the growth of these monolayers for designing devices with optimal and predictable responses.

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**FIGURE CAPTIONS**

**Figure 1.** Sketches of the AFM methods used in this work to characterize thiolated ssDNA monolayers. (a) Peak Force Tapping™ (PFT-AFM): The tip-sample distance is periodically modulated at 2 kHz in order to acquire force-distance curves at high speed during the sample scanning. The blue and red line represents the force-distance curves during tip approaching and withdrawal, respectively. The maximum tip-sample repulsive force, referred to as peak force the feedback signal to obtain the topography of the surface. In this work, we measure the adhesion obtained during the tip-sample separation. (b) Kelvin Probe mode (KP-AFM): Each line is scanned twice. During the first pass, the cantilever is mechanically excited at near its resonance frequency, and the amplitude is kept constant by a feedback system to obtain the topography. In the second pass, the cantilever tracks the previously recorded height profile at a set lift height, 15-50 nm. The image obtained by recording the DC tip voltage reflects spatial variations of the surface potential along the sample surface.
Figure 2. (a) PFT topography image of a thin gold-film functionalized with the 19 mer ssDNA probe. The image shows a uniform ssDNA monolayer with physisorbed multilayer islands. (b) PFT adhesion image. Independently of the number of layers, the physisorbed islands show uniform adhesion, with the lowest value in the image. The islands of one, two and three layers are labeled as 2, 3 and 4, respectively. The adhesion map also reveals that the ssDNA monolayer exhibits two phases. The phases of lower and higher adhesion are labeled as 1 and 1', respectively. (c) Histogram of heights from the image shown in (a). It shows that the islands have a thickness that is a multiple of 4.5±0.5 nm. (d) Histogram of the adhesion values found in the image shown in (b). It shows three levels of adhesion, one for the physisorbed islands, and two for the monolayer.
Figure 3. (a) PFT topography of a 5x5 µm² region of polycrystalline gold functionalized with a ssDNA SAM. (b) Adhesion map obtained simultaneously to the topography in (a). Two domains with different adhesion arise that are not visible in the topography image. (c) Histogram of the adhesion values in (b) and bimodal Gaussian distribution fit. The ratio between the adhesion positions of the two peaks is 1.3. The 500x500 nm² region marked with a white discontinuous line is shown in figure 4.
Figure 4. High resolution imaging of the area marked in figure 3 with a white discontinuous line. (a) PFT topography image showing the typical grain structure of the polycrystalline gold. The grain size ranges from 50 to 80 nm and many of the grains exhibit small height depressions near the grain center that are typical structural defects in SAMs. (b) Adhesion image revealing two different phases. The domain with lower mean adhesion shows uniform values of adhesion, while nanoscale adhesion heterogeneities are observed in the domain with higher mean adhesion. (c) Height and adhesion cross-sections across the domain boundary following the dotted line in (a) and (b). Remarkably, in the domain of higher mean adhesion the intergrain regions provide significantly higher local adhesion than in the grain top, whereas this adhesion contrast is not found in the domain of lower mean adhesion.
Figure 5. (a) Tapping mode topography of a thin gold layer functionalized with thiolated ssDNA AFM showing the typical grain morphology of polycrystalline gold with no evidences of phase segregation. (b) Surface potential image showing two domains similar to those revealed by adhesion in the PFT mode. The region of more negative potential is attributed to a higher DNA density. A high resolution image of the boundary region marked here with a white dotted line is shown in figure 6.
Figure 6. High resolution imaging of the area marked in figure 5 with a white discontinuous line. (a) Tapping mode topography that shows gold grains with depression defects near the grain center. (b) Surface potential image that shows two phases with higher and lower DNA density (darker and brighter, respectively). (c) Tapping mode phase shift image. The phase shift image correlates to the surface potential image, but it provides higher spatial resolution due to the lower mean separation between the tip and the surface. Similarly to the adhesion images, the phase image exhibits nearly uniform phase in the region of more negative surface potential, whereas shows nanoscale heterogeneities in the region of less negative surface potential. (d) Topography (top) and phase shift (bottom) across the lines $L_1$ and $L_2$ marked in (a).
Figure 7. (a) Topography image obtained in tapping mode and presented in three dimensions. The colour intensity map is determined by the phase shift. (b) Schematic model derived from our AFM study. The phase shift relates to the packing density and DNA conformation. In the domain of higher packing (area I in (a)), the grains show uniform levels of DNA packing density. In the domain of lower packing density (area II in (a)), the DNA probes preferentially self-assemble on the top of the grains.