Technical paper

Preparation of Atomically Flat Gold Substrates for AFM Measurements

Uroš Maver,[1,*] Odon Planinšek,[2] Janko Jamnik,[1] Abdelrahim Ibrahim Hassanien[1] and Miran Gaberščček[1]

[1] *National Institute of Chemistry, Hajdrihova ulica 19, SI-1000 Ljubljana, Slovenia*

[2] *Faculty of Pharmacy, University of Ljubljana, Slovenia*

** Corresponding author: E-mail: uros.maver@ki.si*
Phone: +386 1 476 0316

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Abstract

Sample preparation is the most important part of a successful measurement with an atomic force microscope (AFM). While various kinds of substrates are used for that purpose, atomically flat gold proved to possess some advantages, namely chemical inertness against oxygen, stability against radicals and suitability for formation of self-assembled monolayers (SAMs) of organic alkanethiols. Fast and simple preparation procedures to achieve quality atomically flat gold substrates are necessary to achieve reproducible results in high resolution imaging. Here we report an improved technique to produce atomically flat gold in a reliable way. We demonstrate its use on the example of high resolution imaging of single walled carbon nanotubes as test molecules.

Keywords: Atomically flat gold, flame annealing, atomic force microscopy, single walled carbon nanotubes

1. Introduction

Technological advances over the past years have raised the bar of scientific requirements regarding the proof of material or phenomena novelty. The latter is especially true in the field of pharmaceutical sciences, where each day several new health affecting problems appear, which require thorough understanding of the underlying processes at the molecular level.

The introduction of nanotechnological methods into health-related research has made two things possible: extended control over the preparation of many pharmaceutical formulations and the development of sophisticated assays to improve the insight into material properties.[1,2]

No incontestable data can be gained without thorough understanding of the slightest changes in the experimental setup. For this reason, introduction of model systems into first stages of research seems a logical step to increase the significance of the results.[3]

AFM has emerged as one of the most suitable techniques to study nanoscale phenomena and has been extensively used over the last couple of years to study several phenomena in biomedical sciences[4]. But as in all other high-tech methods, no good measurements can be made without a clean and quality sample preparation.

Under the term quality sample, we understand samples, which, without any doubt, can yield information about the desired species. When using a high resolution technique like AFM, we have to be very careful not to confuse the information about the desired species with the substrate characteristics[5]. That is why atomically flat surfaces, apart from mica, which is commonly used for much longer, were introduced a couple of years ago, when researchers realized that not all of the data they gathered corresponded to actual species' properties, but were in fact more related to the substrates' characteristics.[6] Atomically flat surfaces are free of surface roughness and proper choice of an inert material for their preparation makes it possible to gather reliable high resolution data after desired sample attachment.[7]

Due to its chemical inertness against oxygen, its stability against radical and a long history of use, gold is one of most used substrates for preparation of AFM-viable flat surfaces. Gold is additionally rather chemically inert to some biomacromolecules, while on the other hand, it is

accessible to chemisorption via the formation of stable gold-thiol bonds with proteins, comprising amino acids methionine and cysteine.[8,9] The abundant knowledge about gold behavior under different circumstances serves as a perfect base for modern preparation of atomically flat gold terraces, particles etc.[10,11]. Atomically flat gold surface has already proved to be suitable for high resolution sample examination like in the case of So et al., where such substrates allowed for molecular recognition of gold-binding peptide.[12]

Several techniques have been introduced in the past to prepare atomically flat gold surfaces.[6,13–22] These techniques differ in regard of the size of the prepared atomically flat regions, the equipment used, and the preparation parameters (like time of preparation, temperature, pressure, initial gold form etc.).

Although several of the previously introduced techniques enable the production of atomically flat surfaces,[13–22] some of them even in large dimensions,[6,20] they mostly experience drawbacks, like for example longer preparation times, the necessity to use costly equipment or the lack of possible procedure tuning.[6,13–22] Therefore, our work focused on the preparation of a fast, cheap and reliable method of atomically flat gold production with the possibility to easily tune the diameter of the final flat surface. Such characteristics are often desired for frequent high resolution AFM measurements, which are almost commonly needed in modern research. The proposed method is tested on the example of the attachment of single walled carbon nanotubes (SWCNT) onto the prepared gold surface. SWCNT exhibit all the characteristics (small diameters and big lengths, folded structure that exhibit small roughness changes, possible crossings of different agglomerated tubes…) that enable the reliability testing of the quality of surface flatness in the sense that enables high resolution imaging.

2. Experimental

2. 1. Cleaning of Equipment

Sample and equipment cleanliness is important for all types of scientific research, especially when studying properties at the molecular level, where contaminants severely alter the final result. If cleanliness is important for other research efforts, it is even more essential for AFM to obtain atomically flat gold terraces. That is the reason for extreme measures regarding the sample preparation and equipment cleaning procedures, which were introduced in our case. For all preparation procedures only high grade nitrogen (5.0) was used, which was additionally passed through a 0.2 μm PTFE filter. After cleaning of all the glassware used for the procedures in deionized water and organic solvents (ethanol, acetone and dichloroethane successively), an additional cleaning step was introduced, in which a boiling nitric acid solution was used for cleaning of all glassware. This procedure was followed by thorough rinsing with milliQ water (water that has been purified and deionized to a high degree (typically 18.2 MΩ cm) by a water purification systems manufactured by Millipore Corporation, USA) and drying under nitrogen. The tweezers used to hold the substrates were cleaned by sonication in an acetone solution, followed by rinsing with milliQ water and drying under high grade nitrogen.

2. 2. Flat Gold Preparation Procedure

2. 2. 1. Preparation of Gold Films on Mica

For all experiments, grade V-1 Muscovite mica (SPI Supplies, USA) was used as the substrate, while high quality gold (99.999%, ALFA AESAR) was used for the evaporation process. The preparation procedure of gold films was as follows: freshly cleaved mica slides (15 × 15 mm) were mounted on a SEM sample holder under ambient conditions with a double sided carbon tape. To exclude as much moisture as possible, the sample holder with the attached mica piece was put into a desiccator and continuously flushed with nitrogen. The as prepared samples were left in the desiccator in a closed nitrogen flooded environment until used. For the evaporation procedure, samples were rapidly transferred to the evaporation chamber, which was then pumped down to a pressure below 4 Torr. After heating the crucible to allow a stable gold evaporation rate of 0.5 Å s^{-1}, an aluminum shutter between the crucible and the sample was removed and a 200 nm thick gold film was evaporated. The thickness of the film was monitored by a quartz crystal resonator. During the evaporation process all samples were rotated to avoid heterogeneity. Immediately after the evaporation all samples were again put into the desiccator, which was flooded with nitrogen for short times or argon, when the sample weren't used for a couple of days. The storage method was already introduced by Nogues et al.[18] and is necessary to exclude diminishing of samples' quality as exposing the sample to air for only several hours mostly results in decreased flat terrace sizes.

2. 2. 2. Annealing Procedure

As the base preparation procedure for our research efforts we used the annealing technique proposed by Nogues et al.[18]. We additionally improved and upgraded their method to yield the best possible substrates, needed for our research efforts of high resolution imaging. Major improvements were made to several parts of their method; most notably we optimized the protocol to use hydrogen instead of Bunsen flame and used several thermo-couples to avoid temperature fluctuations, which could cause inhomogeneous annealing. Our optimized experimental setup allows precise temperature control, higher degree of purity and easy handling. The equipment used in our approach is depicted in Figure 1.

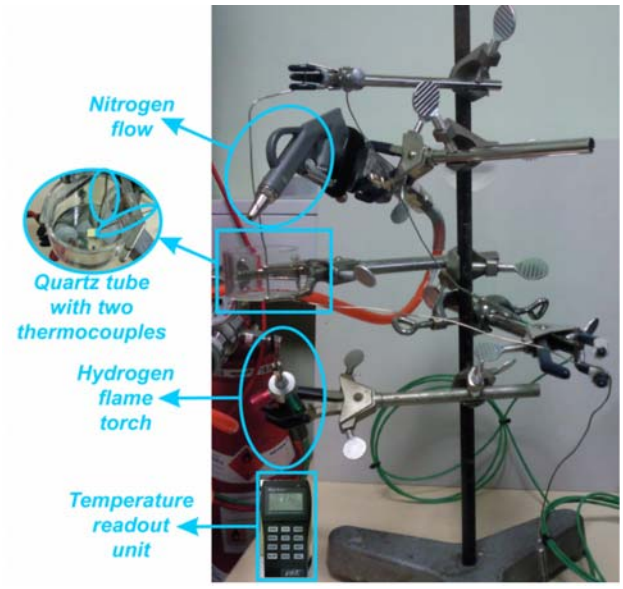


Figure 1: Image of the used experimental setup with explanation of the most crucial parts. Nitrogen and hydrogen cylinders are not shown on the image.

All the improvements were made while keeping in mind a daily routine use of the procedure and its reliability. Being aware that tuning of the preparation parameters will certainly be necessary, we introduced for the flame annealing step, a hydrogen flame torch (Smith Little Torch Jeweler's Torch; Smith, USA). The latter is routinely used by goldsmiths to form jewelry, because of its exceptional tuning capabilities of the flame diameter and temperature. It has also a twin output to add oxygen if extremely high temperatures are needed.

The scheme of our proposed multistep flame annealing procedure is shown in Figure 2.

Prior to the annealing procedure, additional pretreatment of the substrates had to be performed. First the gold coated mica samples were rinsed in milliQ water and dried with nitrogen. Immediately after the drying, the samples were passed, gold face up, in a motion of 1 s, 3-times through the hydrogen flame, which was first left to burn for several minutes to burn all the dirt that could possibly get attached on the flame torch, while it was not being used. The procedure of rinsing and passing through the flame was repeated several times until the gold surface was completely wetted by water, which is a known indication of surface cleanliness.[23] Following the pretreatment phase, the substrate was placed into the bottom of a quartz tube. The substrate was left there for 2 minutes at a flame temperature of 850 ± 20 °C, which corresponds to the tube bottom temperature of 700 ± 10 °C. The specially designed experimental setup and the corresponding procedure scheme are shown in Fig. 2. The procedure can be summarized as follows: a quartz tube (5 cm in diameter, 3 cm high and with a wall thickness of 2 mm) was held over a hydrogen flame, while the tube was continuously flooded with nitrogen at a controlled flux of ≈2.0 L s^{-1}, which had to be adjusted not to cool down the substrate and was therefore positioned as shown in Fig. 1. The position of the nitrogen tube above the flame was aligned to allow the temperature to equilibrate at 700 ± 10 °C at the inside of the tube and 850 ± 20 °C near the outer bottom of the tube, as measured using two separate pre-calibrated thermocouples with the same readout unit. After two minutes of flame exposure at the mentioned temperature, the flame torch was lowered to adjust the temperature to equilibrate

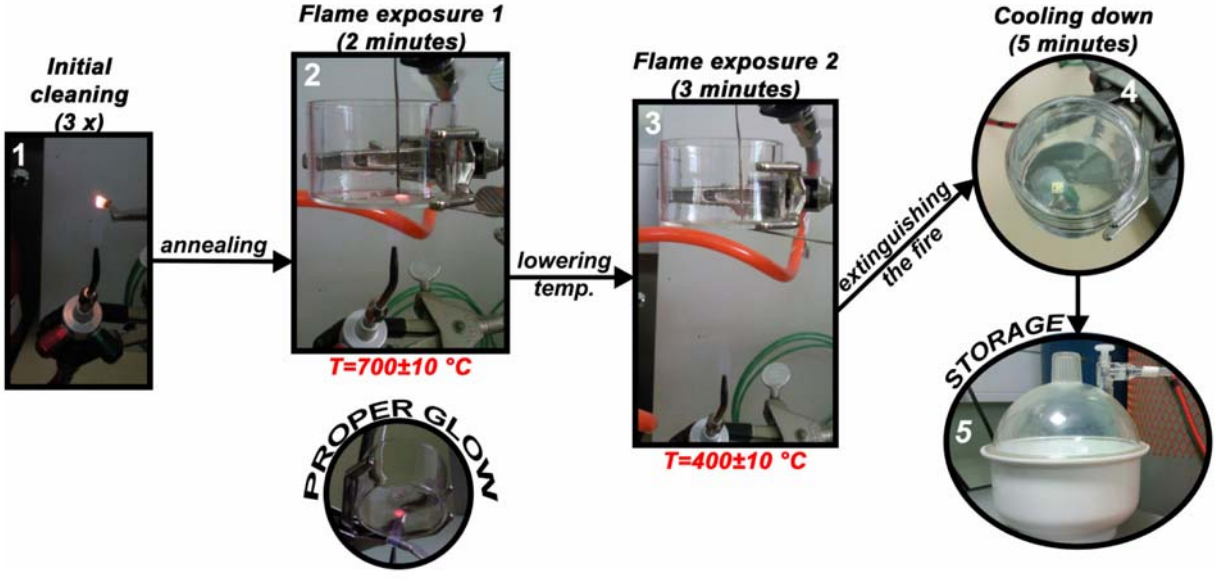


Figure 2: Scheme of annealing procedure with corresponding photographs. The initial cleaning step comprises three passes of the gold coated mica piece through the hydrogen flame. In the "flame exposure 1" step, the temperature was adjusted, which resulted in a certain glow, which can serve as a non-objective parameter for adjusting the temperature even without looking at the readout unit. After the "cooling down" step we either used the substrates right away for attachment of test molecules or stored them in a desiccator in an argon atmosphere as shown above.

at 400 ± 20 °C in the tube interior (corresponding to 550 ± 30 °C) and kept at that distance for additional 3 minutes. The flame torch was then extinguished and the substrate was allowed to cool down for 5 minutes, while the quartz tube was closed. The substrate was then immediately used for the attachment of the desired species or stored in a desiccator unit, flooded with argon.

2. 3. Fixation of Test Molecules (SWCNT – Single Walled Carbon Nanotubes)

For the fixation of test molecules onto freshly prepared atomically flat gold substrates, all the equipment used in the preparation was first thoroughly cleaned as written in the above cleaning section. Because some additional cleaning steps were introduced, we rewrite the procedure here again. SWCNTs were initially in the form of bigger agglomerated chunks, as bought from NanoIntegris.

The flask of 25 ml was cleaned in a three step cleaning procedure to assure the best possible purity of the flask. Such thorough cleaning was introduced to avoid the presence of any possible residues form the outside, which was present if we used only water-based cleaning procedures. Ethanol and acetone were therefore used to get rid of any organic species, while dichloroethane was used as the final solvent due to two reasons, it is namely one of the best possible solvents for suspending CNT and it can be bought as a HPLC standard solvent with extremely high purity. The preparation procedure of the SWCNT suspension was as follows:

Washing of the glass flask:

1. three times deionized water solution, containing a surfactant,
2. three times washed with deionized water,
3. three times washed with milliQ water,
4. washed with ethanol and dried,
5. washed with acetone and left the flask half empty in a sonic bath for 30 minutes,
6. washed with dichloroethane (DCE) and left half empty in a sonic bath for 30 minutes,
7. washed again with DCE.

Suspending of SWCNT:

8. poured 10 ml of fresh high grade DCE into the flask,
9. cut a tiny piece of bulk SWCNT (amount equal to the edge of the rounded tweezers end) and put it into the flask,
10. as prepared suspension of SWCNT was put into a sonic bath for 45 minutes (or until a blackish transparent suspension was achieved without any visible particles),
11. the suspension was thoroughly sealed with a glass cap, covered with parafilm and stored in a refrigerator at 4 °C until use.

Prior to addition of the prepared SWCNT suspension onto the freshly prepared atomically flat gold pieces, the suspension was shaken up with the Lab dancer (Vortex). Two drops of 10 µl were poured onto the as prepared gold substrates, left there for approximately 30 seconds to leave the tubes some time to attach, washed three times with isopropanol of high purity and dried with high purity nitrogen (grade 5.0). Dried samples were immediately put either under the microscope for examination (controlled environment flooded with nitrogen under AFM or vacuum conditions in the SEM chamber) or into a desiccator, flooded with argon for future examination.

The overall nanotubes attachment procedure is depicted in Figure 3.

All chemicals were bought from either Sigma-Aldrich or were prepared and purified in laboratory to assure the highest possible purity.

Figure 3: Stepwise preparation procedure of test samples for high resolution AFM imaging.

2. 4. Characterization

2. 4. 1. Scanning Electron Microscopy (SEM)

To obtain information about the samples' quality and morphology scanning electron microscopy (with or without attached test molecules) was used. Prior to imaging all samples were pressed on a double-sided adhesive carbon tape (SPI Supplies, USA) and then imaged using a field emission scanning electron microscope (FE-SEM, Supra 35 VP, Carl Zeiss, Germany) operated at 1 keV. Several magnifications were used to get as much information about the samples' morphology as possible.

2. 4. 2. Atomic Force Microscopy (AFM)

As prepared samples (with or without attached test molecules) were mounted onto round shaped metal discs and mounted on the micrometer positioning stage of the Multimode V AFM (Veeco Instruments/Bruker). Tapping Mode AFM mode was used to simultaneously acquire height, phase, and amplitude images. Silicon AFM tips doped with antimony (FESPA tapping mode tips, Bruker) with a nominal spring constant of k = 2.8 N/m were used for imaging purposes for all samples. The typical free vibration amplitude was in the range of A = 25 nm or lower for samples with attached SWCNT. For all samples images of 4 × 4 μm^2 were recorded with a resolution of 2048 × 2048 pixels. All images were processed and the corresponding roughness was calculated using WSxM software.[24]

3. Results and Discussion

3. 1. Atomically Flat Gold Preparation Procedure

The relatively simple and very fast preparation procedure (especially when using purchased gold coated mica), proved to be very reliable to produce substrates with atomically flat regions, satisfying most applications for study of characteristics or structure related features at the nanoscale with nanotechnological methods like AFM. The success rate of the preparation procedure was estimated to be 9 out of 10 due to the possibility of human error in handling the experimental setup. High success rates are required because routine preparation procedures should not hold back the actual measurements. Figure 2 shows the photographs of the different stages of the preparation procedure. It can be clearly seen that the actual annealing procedure does not exhibit any danger to the researcher or the nearby environment and requires only slight handling, but the initial cleaning procedure comprises a step using boiling nitric acid, which is an extremely aggressive solution, therefore extreme care has to be taken during its use. Most importantly, the preparation procedure assures cleanliness of the equipment used and the thorough follow-up to the mentioned parameters. Also the success rate can be kept very high for a prolonged period of time. Special focus has also to be put on the storage of the prepared substrates as their quality drastically decreases with expo-

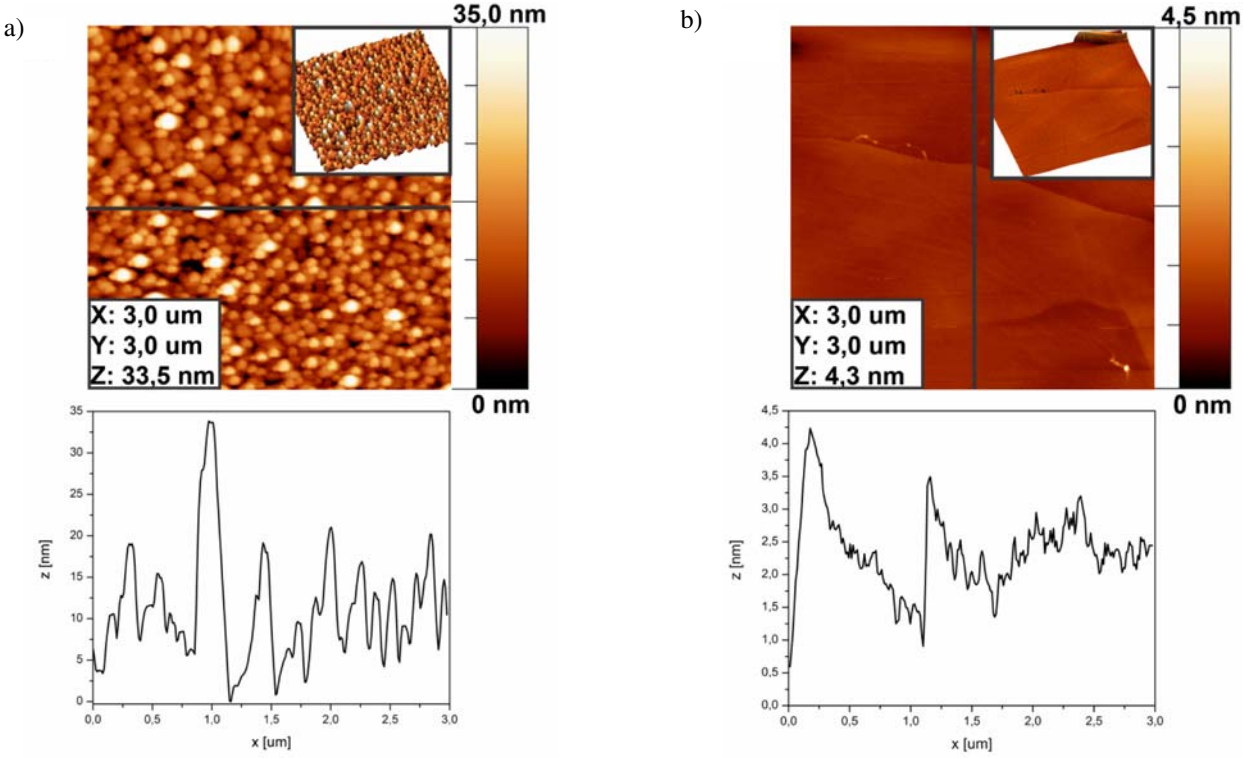


Figure 4: Comparison of AFM images a) before and b) after annealing with corresponding profiles, which were taken at the drawn line. Additionally 3D images are shown in the upper right corners.

sure to air. Small desiccators proved to be very suitable for the storage of not immediately used substrates under a controlled argon atmosphere.

To test the suitability of the prepared substrates for a high resolution examination, SWCNTs were deposited on the prepared substrates. Small molecules such as SWCNT require a flat surface to be thoroughly examined beyond doubt about the measurements authenticity.

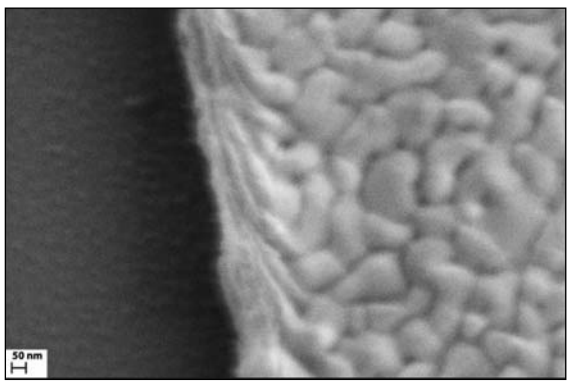
3. 2. Characterization

Each of the preparation procedure steps was examined using SEM and AFM microscopy.

3. 2. 1. Analysis Before SWCNT Attachment

AFM images of samples prior and after annealing and the corresponding roughness profiles across the sample surface are shown in Figure 4. It can be clearly seen that the initial samples exhibit a granulate surface with a grain size of 20–40 nm. After the annealing procedure, the grains have disappeared, while newly formed terraces covered the mica surface. The size of these terraces is variable due to the fact that the flame temperature radially decreases from the center onward, meaning that not all regions are exposed to the same flame temperature, which leads to their various sizes. Typical diameters of the formed terraces are in the range of several hundreds of nm to several microns, while the surface roughness is indeed below 1 nm (Figure 4b).

a)



b)

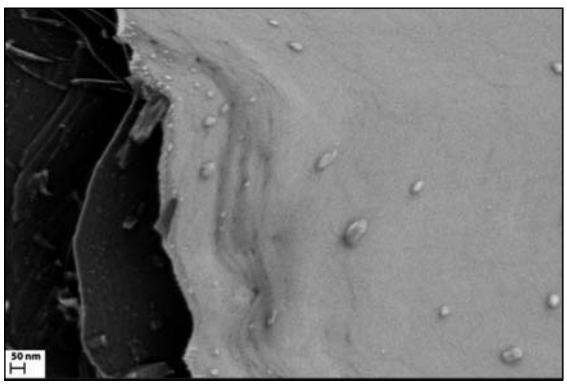


Figure 5: SEM micrographs of susbstrates a) before and b) after annealing. Grains can be clearly observed in the substrate before annealing as shown in the micrograph, noted as a. The micrograph in b for the sample after annealing exhibits terraces grown on each other. No other morphological features can be observed in the annealed sample.

One of our goals was to find an easy way for evaluation of the proposed technique. We decided to use SEM, which is one of the most common techniques for initial sample characterization. Figure 5 shows the SEM micrographs for the samples prior and after the annealing procedure. Again there is a clearly observable increase in the samples' flatness after the exposure to the flame.

3. 2. 2. Analysis After SWCNT Attachment

The final test of the suitability of the proposed atomically flat gold preparation procedure is an actual high resolution measurement of test molecules on such substrates. SWCNT were chosen, because of their relatively small size, especially considering the diameter close to 1 nanometer. SWCNT are also interesting due to the fact that different SWCNTs and their functional derivatives have been proposed for novel drug delivery systems.[25]

Figure 6 shows the results from AFM imaging of the samples after attachment of SWCNT with the measured profiles attached below the images. A couple of SWCNT concentrations were used to show the suitability of the substrates for imaging of differently populated surfaces. The recorded profiles show the flatness of the prepared substrates for regions free from molecules, while at points where the test molecules appear, sharp peaks arise. This result is a clear indication of the suitability of the proposed substrate preparation procedure for the attachment and examination of small molecules. Figure 6 also shows images of attached SWCNT on non-annealed surfaces. Although the shown region exhibits only a few attached molecules (several other regions, like the one shown in Figure 7, are covered with tubes), it is evident from the measured profile that no clear distinction between the grains and tubes can be made. Such a surface roughness prevents a thorough examination, especially of the structural characteristics in small species like the carbon nanotubes. Grains could also mask possible surface features like bumps or otherwise observable functionalization. The z scales in Figure 6b and c are higher, when compared to the bare substrates due to a contrasting image processing technique. This takes into account the lowest and highest parts of the image as the boundary heights (the lowest and highest regions appear as black and white, respectively). When looking at the contrasts attached at the sides of the Figures 6b and c, one can deduce that the actual height differences are actually smaller.

Scanning electron microscopy was used again to show the changes in the sample surface after SWCNT attachment. Namely, the substrate flatness allows for clear examination of the attached SWCNTs even under SEM.

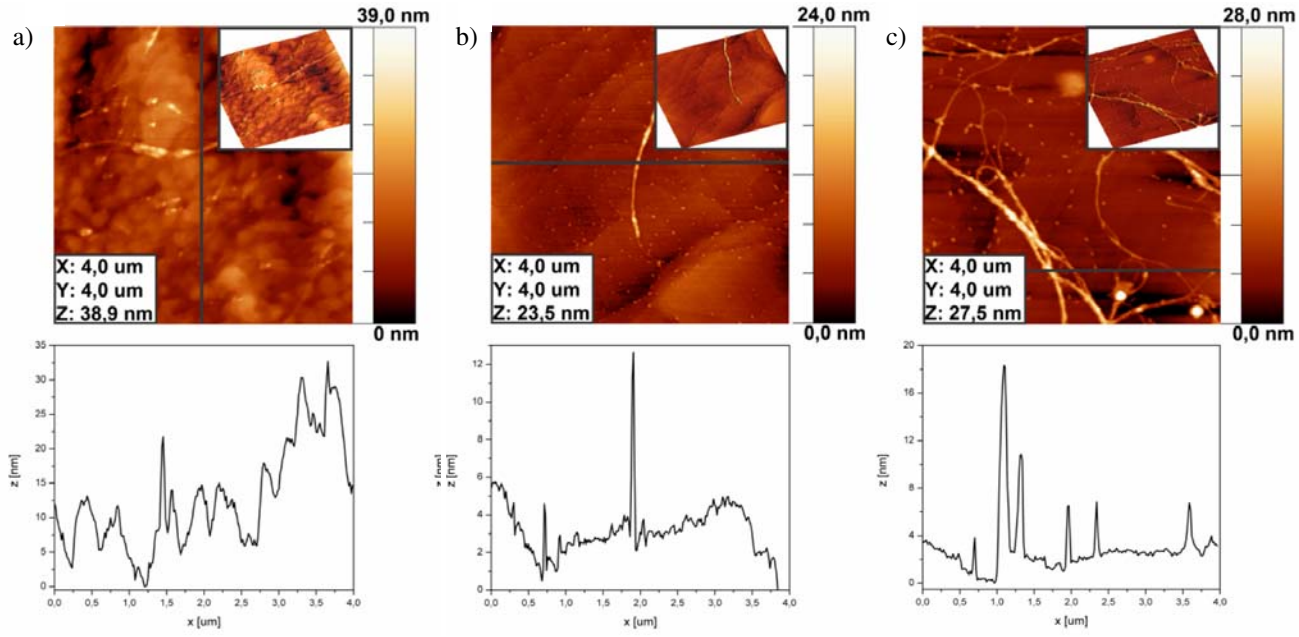


Figure 6: Comparison of AFM images after the attachment of SWCNT to a) not annealed and annealed substrates – b) and c). Concentration of SWCNT was altered for the preparation of sample b) and c), where for the latter an increased concentration was used. Sharp peaks at the points, where the tubes are attached can be observed only for the samples, prepared on annealed substrates. Figures b and c exhibit somewhat higher z scales due to basins and CNT bundling.

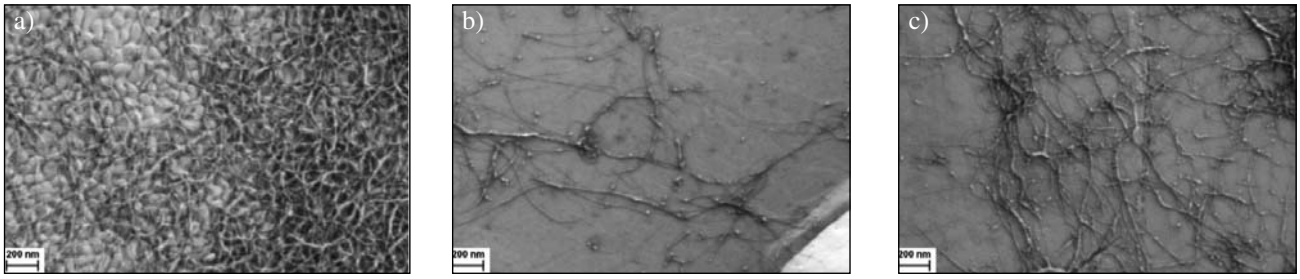


Figure 7: SEM micrographs of samples after the attachment of SWCNT to a) not annealed and b), c) annealed substrates. Annealed samples are clearly more suitable for such attachment and for further examination. A clear distinction between different tubes can be made for both samples, where annealed gold was used as the substrate.

Figure 7 shows the SEM images of the non- and annealed substrates with attached SWCNTs. It is obvious that in a non-annealed substrate (Fig. 7 a) the distribution of molecules is much less suitable for the purpose of single molecule examination if compared to both annealed substrates (Fig. 7 b, c). In non-annealed substrate, the additional features (grains in this particular case) serve as anchoring sites for the attachment of big agglomerates, which cannot be washed away easily after the initial dropping of the SWCNT suspension onto the substrates.

4. Conclusions

We introduced a fast, simple and reliable technique for the preparation of atomically flat gold surfaces. Big terraces of atomically flat gold were produced using the proposed technique, which allow thorough examination of samples at the highest possible resolution. On the example of SWCNT it was shown that in annealed substrates the degree of particle agglomeration is significantly lower than in non-annealed ones. The proposed technique is not only easier to carry out but also more reproducible than the previous methods.

5. Acknowledgements

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Povzetek

Pravilna in skrbna priprava vzorcev je vsekakor osnova za uspešno izvedbo meritev z mikroskopijo na atomsko silo (AFM). Kljub velikemu številu splošno uporabljenih podlag z različnimi morfološkimi in strukturnimi lastnostmi v ta namen, so atomsko ravne površine topogledno vsekakor prevladale. Njihova hitra in relativno enostavna priprava je nujna za zagotavljanje visoko-ločljivostnih meritev z AFM na dnevni ravni. V članku je predstavljena izboljšana metoda za zanesljivo in ponovljivo pripravo atomsko ravnih površin zlata, ki so primerne za slikanje z visoko ločljivostjo. Med največje izboljšave štejemo lažjo kontrolo nad končno velikostjo ravnih površin ter splošna varnost in enostavnost metode, ki vodi do enakih ali boljših rezultatov v primerjavi s poprej uporabljanimi.