

RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

Chemical modification of scanning tunneling microscopy tips for identification of functional groups in self-assembled monolayers

Volcke, Cedric

Published in:

Microscopy: Science, Technology, Applications and Education

Publication date:

2010

[Link to publication](#)

Citation for pulished version (HARVARD):

Volcke, C 2010, Chemical modification of scanning tunneling microscopy tips for identification of functional groups in self-assembled monolayers. in A Mendez-Vilas & J Diaz (eds), *Microscopy: Science, Technology, Applications and Education*. vol. 2, Antonio Mendez-Vilas and Jesus Diaz Alvarez, Formatex Research Center - Spain, pp. 1293-1301.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Chemical modification of scanning tunneling microscopy tips for identification of functional groups in self-assembled monolayers

C. Volcke

Research centre in Physics of Matter and Radiation (PMR), University of Namur (FUNDP), 61 rue de Bruxelles, 5000 Namur, Belgium

Since 30 years, scanning tunneling microscopy (STM) allows individual atoms or molecules to be observed. Amongst others, it is particularly well adapted to image molecular organizations at liquid/solid interfaces as it is already successful in imaging physisorbed self-assembled monolayer (SAM) structures adsorbed on graphite, highlighting a variety of physical, chemical, dynamical and electronic phenomena. In this context, another important aspect of the molecular layers investigation with STM is its ability to discriminate functional groups from the molecule backbone. However, while functional groups such as amines or thiols can be easily distinguished from the molecule skeleton in STM images, others (ester or carboxyl groups for example) can not. During the last decade, several research groups focused on the STM tip chemical modification in order to change contrast of functional groups in STM images. In this minireview, we describe the STM tip modification phenomenon and its effect on STM tunneling current. The different STM tip modification performed are listed and their application to the identification of various functional groups within SAM at the liquid/graphite interface detailed.

Keywords Scanning tunneling microscopy; self-assembly; liquid/solid interface.

Since its discovery about 30 years ago [1], scanning tunneling microscopy (STM) rapidly developed, to almost become essential nowadays to many physical, chemical and even biological laboratories. It relies on tunneling effect as operating principle. Practically, if a conductive tip and a conductive sample surface are close enough to each other (typically only a few angstroms), their electronic wave functions overlap. Upon application of a bias voltage between both electrodes, a current (called tunneling current) will flow, whose direction is defined by the sign of the bias voltage applied [2]. The isolating gap between electrodes can be vacuum, air, liquid or molecules. It has therefore allowed clean and covered surface structures in air or vacuum as well as in liquids to be studied [3-5]. For example, it allowed the understanding of the complex (7x7) reconstruction structure of Si(111) surface [6-8]. Also, organic layers properties were investigated as it is a very important aspect for the fundamental understanding of two-dimensional ordering processes as well as for adhesives and lubrication technology [9]. Molecular arrangements of amphiphilic molecules [10], liquid crystals [11-12] or biomolecules [13-16] at the liquid/solid interface were already successfully analyzed, revealing a high degree of two-dimensional ordering. For example, alkanes adsorbed on the basal plane of graphite adopt an all-trans configuration, with the molecule axes parallel to each other in order to optimize intermolecular and molecule-substrate interactions [17-19]. A number of other substituted alkanes [20-21], long chain ethers [22], fatty acids [23] or benzene derivatives [24-26] were also studied.

In this context, STM has a wider application field than just providing images of surface structures. It is also capable to distinguish some functional groups from the methylene groups of the molecular backbone. However, while functional groups like amines, thiols or halides can be easily distinguished in STM images from molecule skeleton, others like esters or carboxyl groups are not. By remembering the fact that in a STM, if the tip and sample electronic wave functions overlap, a tunneling current can be measured as a bias voltage is applied between both electrodes. This suggests that tip electronic wave function modification can induce tunneling current modification, allowing possible discrimination of chemical species in STM images of the surface.

This fact is first described by Bartels et al. in 1997 [27-28]. While analyzing the possibility to transfer CO molecules from a copper surface onto the STM tip and then back to the surface, they noticed that CO molecules always appears as depressions (independently of the bias voltage polarity) while imaged with a clean STM tip [27]. However, once imaged with a tip presenting a CO molecule at its apex, they appeared as protrusions (independently of the bias voltage applied). This is the beginning of chemical sensitive imaging with STM. They later discriminated CO molecules from oxygen atoms adsorbed on a Cu(111) surface [28]. Indeed, by transferring CO molecules on the tip apex, they yielded a contrast reversal for most of the dark spots while some of them stayed unchanged. They concluded some spots are due to oxygen while others are due to CO molecules. So distinction between different adsorbates was therefore possible using STM modified tips [29-31]. However, the "Bartels et al." chemically sensitive imaging with STM is somewhat limited to ultra-high vacuum conditions. In order to contrivene this issue and to extend this technique to a large variety of molecules physisorbed on substrates, chemically modified STM tips were generated and applied to the analysis of two-dimensional molecular structures at liquid/solid interfaces. This is first described by T. Ito et al [32]. They examined the effect of tip modification on the STM image contrast of -OH and -COOH residues in alcohol and fatty acids self-assembled monolayers (SAMs) at the phenyloctane/graphite interface. The tip modification procedure used self-assembled monolayers of thiols derivatives on gold tips. Their results suggested that such chemical modification of STM tips allow one, based on hydrogen bond interactions, to recognize functional groups inside SAMs.

In this review, we'll describe the literature in this domain. The chemical contrast STM imaging phenomenon is described and its interpretation explained. Then tip modification procedures are detailed and their effect and advantages on STM image contrast (in terms of resolution enhancement, chemical contrast recovery) demonstrated. Further, the different scientific advances of such a technique are listed, i.e. functional groups identification and orientation determination... Also, some particular observed phenomena are reported such as molecular dynamics at interfaces or multilayers formation. Finally, the latest developments are described.

1. Chemical contrast and interpretation

1.1. Chemical contrast

The identification of functional groups in SAMs of substituted hydrocarbons was first demonstrated by Ito et al. while using modified STM gold tips [32]. STM images of substituted hydrocarbons observed with clean unmodified tips were extensively already detailed in literature [17-19]. For examples, lamellar organization of alcohols molecule SAMs at liquid/graphite interface were observed (Figure 1a) [32]. These lamellae, separated from the adjacent one by dark borderlines, are consisting of parallel bright bands, associated to the alkyl chain of the molecule (as deduced from geometric observations). Inside a lamella, the alcohol molecules are packed parallel to each other with their molecular axes oriented at 60° relative to dark lamella borders. However, the position of the alcohol functional groups could not be deduced based on such images.

Fortunately, it was possible to recognize –OH residues from molecular backbone by using modified STM gold tips as observed in Figure 1b [32]. In this case, a sharp gold wire used as STM tip is dipped for 12 hours in a 1-10 mM ethanolic solution of 4-mercaptopyridine (4MP). The thiol groups are bonding to gold wire surface, leaving pyridine residues in contact with the external solution. The STM images obtained on alcohol molecule SAM using such modified STM tip reveal parallel bright lines. These lines are associated to hydroxyl functional groups, which are the only reactive parts of the molecules [32-33].

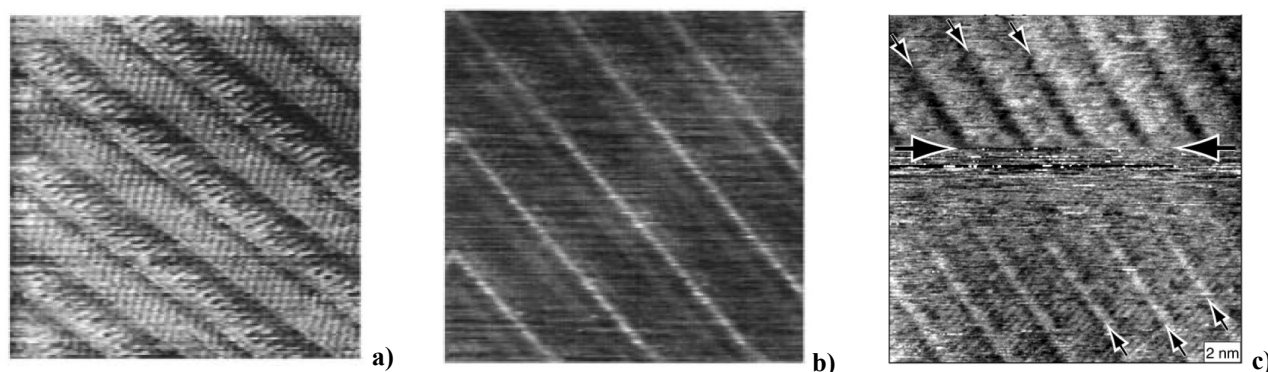


Figure 1. STM images of 1-octadecanol physisorbed at the 1-phenyloctane/graphite interface, as obtained with (a) an unmodified gold tip (-1.0V, 1nA) and (b) a 4-mercaptopyridine-modified gold tip (-1.0V, 0.7nA). Images size: 20 nm x 20 nm. Reprinted with permission from Ref [32]. Copyright 1998 American Chemical Society (c) STM image of 1-octadecanoic acid SAM at the the 1-phenyloctane/graphite interface showing the reestablishment of chemical contrast enhancement. Initially, no chemical contrast is visible (top part) as carboxyl groups appear as dark spots. After a bias pulse of +1.9 V during 15 sec inducing tip instability (central area), the contrast enhancement is observed (bottom part), indicating the presence of 4MP molecule on tip extremity. Reprinted with permission from Ref [34]. Copyright 2003 American Chemical Society

1.2. Interpretation

Remembering the basics of STM operation, if a conductive sample surface and tip are close enough to each other, their electronic wave functions can overlap. Upon application of a bias voltage, a so-called tunneling current can flow between both conducting electrodes [2]. Therefore, just like in electrochemistry, the overlap of electronic wave functions is a requisite for electron transfer. This suggest that controlled modification of STM tip should induce electron transfer modifications and therefore variations in STM image contrast. As demonstrated previously by Bartels et al. [27-28, 30-31], adsorption of atoms on a STM tip changes tip electronic structure and electronic states, but also STM images contrast as a consequence.

Here, it is expected that the enhanced contrast observed on –OH residues in organic molecules SAM while using modified STM gold tips is due to electron transfer through hydrogen bonds between the functional groups on tip (pyridine) and sample (hydroxyl). As electron transfer by tunneling through hydrogen bond interactions has attracted considerable interests [35-37], studies have shown that electron coupling modulated by hydrogen bonds can be larger than through σ bonds [36]. If applied to the STM configuration, it should enhance contrast while hydrogen bond is

formed between tip and sample surface. We can therefore suggest that the enhanced contrast observed in Figure 1b are due to the hydrogen bond interactions between functional groups on tip (pyridine) and sample (hydroxyl), which is known to favour electron tunneling.

To demonstrate this assumption, Ito et al. used different molecules to functionalize the STM tip [32, 38]. They examined how tip modification with various molecules affects images of octadecanol SAMs at liquid/solid interface. The molecules used to modify the gold tips are thiophenol (TP), 4-mercaptopyridine (4MP), 4-aminothiophenol (4ATP) and 4-hydroxythiophenol (4HTP) [32]. Their results seem to reflect the hydrogen bond basicity of the functional groups in the thiol derivatives used for tip functionalization. Whereas enhanced contrast were observed with tips modified with 4MP or 4ATP (having strong hydrogen bond basicity), the small effect of tip modification with 4HTP and the absence of effect of tip modification with TP seem to be the result of the small hydrogen bond basicity of their functional groups [32]. These confirms the hypothesis that the enhanced contrast in these STM images arise because of the presence of hydroxyl residues on surface and are due to the hydrogen bond interactions between functional groups on tip and sample.

2. Tip modification effect and observed phenomena

The STM tip modification has several effect on imaging. As above-mentioned, the appearance of a chemically sensitive image contrast is one of the most obvious one. However, another effect was also observed : resolution enhancement, as described in the first part of this section. Further, tip apex structures can be unstable during imaging, leading to tip apex structure reorganization, inducing loss/recovery of image resolution. This also happened while using modified STM tips. This effect can also be associated to molecule extraction from apex, leading to the loss of the enhanced chemical contrast. A procedure described in a second part of this section was developed to contrivene such issue. Finally, the last part of this section describes processes and phenomena routinely observed using unmodified STM tips also highlighted using modified tips.

2.1. Resolution and contrast enhancement

The immediate observation following tip modification is a resolution enhancement, certainly due to a decrease in tip radius of curvature. For example, several studies pointed out the difficulties in imaging esters molecules at the phenyloctane/graphite interface [39-40]. In STM experiments on wax esters with standard tips, submolecular resolution could never been reached although a considerable amount of trials. Oppositely, with functionalized gold tips, it was easily possible to observe submolecular resolution on the same layers. STM images revealed better contrast and image resolution [39-40]. Of course, the second obvious phenomenon, described above, is the contrast enhancement while hydrogen bond is formed between tip and sample surface.

2.2. Recovery of chemical contrast

While performing chemical sensitive STM imaging, unfortunately only a few portion of modified tips are providing a contrast enhancement. For example, 19 % and 31 % of STM tips modified with 4MP and 4ATP, respectively, are providing contrast enhancement as calculated by Ito et al [32]. This low ratio may be related to difficulties in controlling tip shape and/or thiol molecules tip coverage. Indeed, Such small size mercaptan molecules induce weak lateral interactions in SAMs. This lead to an assumed low SAM stability and poor organization. Moreover, contrast enhancement can also be lost after several images. This can be due to removal of molecules from the tip apex, contaminations on tip and/or tip shape modification during scanning. Ito et al. expected the enhanced contrast to be regenerated by tip immersion in thiol solution. It was however later demonstrated by Olson and Bühlmann [34] that chemical contrast enhancement can be (re)generated in situ. The procedure developed takes advantage of the 4MP molecules mobility still present in the surrounding of the tip apex, even when exhibiting no contrast enhancement. By moving those molecules towards the tip apex, the chemical contrast enhancement can be (re)established. To achieve it, the imaging condition, precisely the bias voltage applied between tip and surface, is adjusted. Indeed, the electric field at tip apex, due to a high voltage pulse, may induce a lateral movement of the 4MP molecules towards the apex. Such displacement is consistent with the molecule dipole moment. If a positive bias voltage pulse is applied, 4MP molecules will be attracted to the tip apex. Oppositely, by applying a negative bias voltage pulse, extraction of 4MP molecules from the tip extremity is expected.

As an example, carboxylic residues in octadecanoic acid SAMs at the phenyloctane/graphite interface were imaged using 4MP modified gold tips (Figure 1c). It started from an STM image obtained with 4MP modified tip in which the chemical contrast is not visible or has been lost during imaging. The resulting image is highlighting dark spots rows (Figure 1c , top part), similar to characteristic features observed with unmodified tips [32, 41-42]. In the middle of the image, a positive bias pulse of +1.9 V is applied for 15 sec, causing distortion observed in the central area. Then the bias was decreased to its original value. The lower part of the image, obtained after the treatment, shows carboxyl groups as bright spots, i.e., manifesting chemical contrast enhancement. This confirms the presence of 4MP molecules

on tip apex. The efficiency of this procedure was also demonstrated by Volcke et al. on palmitic acid SAMs imaged at liquid/graphite interface using 4-mercaptotoluene (4MT) modified gold tips [40].

2.3. Observed phenomena

This section is aimed at confirming that processes and phenomena routinely observed using unmodified STM tips can also be identified with functionalized STM tips. Dynamical processes in SAMs at liquid/solid interface [33, 39] and multilayers formation [33] are such examples revealed in literature.

a. Molecular dynamics

4-mercaptobenzoic acid (4MBA) modified gold tips were reported to provide STM images of molecular dynamics in wax ester [39] and alcohol [33] molecule SAMs at phenyloctane/graphite interface. In wax ester SAMs image, two domains composed of lamellae oriented in the same direction separated by a non structured gap are initially revealed. While successive imaging is performed, the gap between domains get progressively structured: lamellae are appearing in the non-structured gap, tending to align on lamellae of the adjacent domains to finally form one unique and continuous domain. The direction of the lamellae constituting the gap area are tilted around 60° related to the direction of lamellae in adjacent areas. Finally, all lamellae tend to progressively adopt the same direction. This was the first observation of the birth and evolution of junction between two ordered domains in wax ester SAM. This process, routinely observed in self-assembled monolayers physisorbed at liquid/solid interfaces can be understood as a progressive disappearance of small domains while larger domains grow, a phenomenon known as *Ostwald ripening*. Moreover, as the enhanced contrast is visible in all lamellae, this indicates the characteristics observed with functionalized STM tips, i.e., the enhanced contrast, are independant of the scan direction [39]. Similar observations and conclusions were obtained on alcohol molecule SAM [33].

b. Multilayers

Bilayer structures of wax ester molecules were also observed at the liquid/solid interface. Such as the underlying (bottom) layer, the top layer appears to be formed by parallel sticks, oriented in the same direction as molecules in the bottom layer. Moreover, the top layer is sliding over the bottom layer surface composed of organized molecules, probably pushed by the STM tip. Further, the top layer is located at a grain boundary of the bottom layer, as lamellae in this layer on both sides of the upperlayer are shifted vertically. This suggest that grain boundaries constitutes defect sites which promotes the formation of bilayer structures [33].

3. Scientific advances reported

This chemical sensitive imaging technique allowed scientific advances. Indeed, the fingerprint of several functional groups, not visible in STM images otherwise, were identified inside self-assembled monolayers of molecules imaged at the liquid/solid interface. Precisely, hydroxyl [32-33], carboxyl [32, 41], ether oxygen [43-44] and ester [39, 40, 45] residues were observed using modified gold STM tips. These are described in the first part of this section. Moreover, advanced investigations were revealing the orientation of ether oxygen [43-44] and ester [39] functional groups related to the surface plane, as described later in this section.

3.1. Chemical groups identification

a. Hydroxyl (-OH)

The first functional groups identified through this technique were hydroxyl residues in alcohol molecules (1-octanol) self-assembled at the phenyloctane/graphite interface [32]. These molecules, as already described above, structures in lamellae composed of bright parallel bands being separated from adjacent lamellae by darker borderlines (Figure 1a). The bright bands were associated to the octadecyl hydrocarbon chain physisorbed on the graphite surface, as confirmed by their dimensions. The molecules are packed parallel to each other inside lamellae with their molecular axes oriented around 60° related to dark lamella borders. The -OH end group can not be distinguished from the hydrocarbon backbone, while using unmodified STM tips. However, once imaged using tips modified with 4MP, parallel bright lines separated by twice the molecule length are observed (Figure 1b). This image clearly indicates the position (i.e. bright lines) of the hydroxyl group, which constitutes the only reactive part of the molecule. These results show that the chemical modification of STM tips allows one, based on hydrogen bonding interactions, to recognize -OH functional groups in monolayers of 1-octadecanol at the phenyloctane/graphite interface. Moreover, this suggest that within a lamella, all hydroxyl groups are pointing into the same direction. Furthermore, the methyl groups of one lamella face the methyl group of an adjacent lamella, while hydroxyl groups form hydrogen bonds to the hydroxyl groups of another lamella. A structural model was elaborated as also confirmed by Volcke et al. [33].

b. Carboxyl (-COOH)

Similarly, enhanced contrast for carboxyl residues in fatty acids self-assembled monolayers (1-octadecanoic acid [32], lauric and palmitic acid [40-41, 45]) onto graphite from 1-phenyloctane solutions was observed with gold modified tips

(Figures 2) [40]. Side-to-side packing of lamellae, formed by a closed packing arrangement of parallel sticks, perpendicularly oriented with respect to the troughs is observed with unmodified tips (Figure 2a) [40]. Bright spots appear in the troughs (i.e., at the carboxyl group position) while imaged using 4MT modified tips (Figure 2b) [40]. This allowed the confirmation of packing model elaborated by Hibino et al. 10 years ago [42].

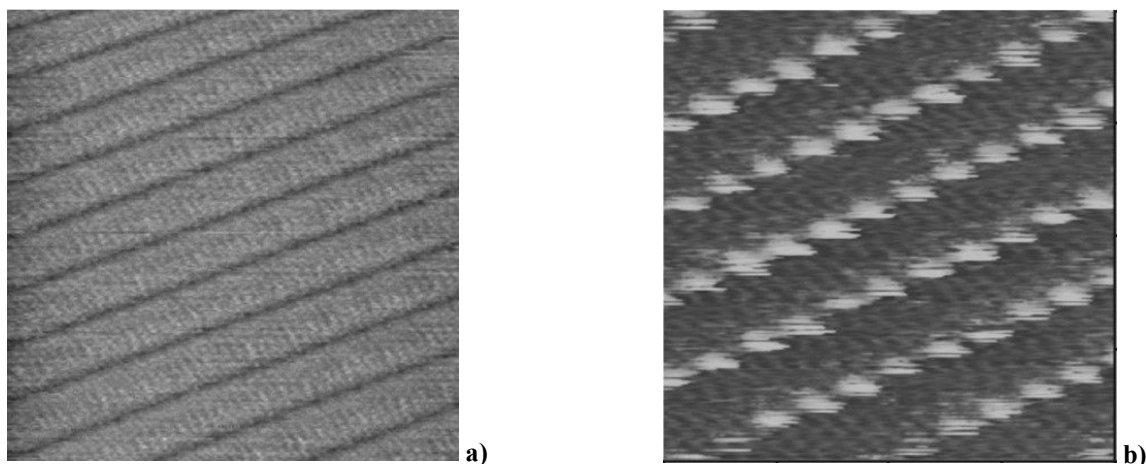


Figure 2. (a) STM images of palmitic acid SAMs at the phenyloctane/graphite interface obtained with an unmodified Pt/Ir STM tip (-480 mV, 500 pA). Image size : 20 nm x 20 nm. (b) STM image of lauric acid SAM at the phenyloctane/graphite interface obtained using 4MT modified STM gold tips (-765 mV, 710 pA). Image size: 10 nm x 10 nm. Reproduced from Ref [41]. Reprinted with permission from Institute of Physics Publishing.

c. Ethers (-C-O-C-)

As a new example of selective contrast enhancement by chemically modified STM tips, STM images of samples presenting hydrogen bond-accepting groups, i.e. ether oxygen, were presented by Nishino et al. [43-44]. In such a case, the tip-modifying molecules (4MBA) need to work as hydrogen bond donor to meet the requisite for hydrogen bond formation with ether oxygen groups in the sample. STM images of a dihexadecyl ether monolayer physisorbed from 1-phenyloctane solution onto HOPG were obtained (Figures 3) [44]. Lamellae, as observed using unmodified tip (Figure 3a), are separated from each other by dark troughs and consisting of bright parallel bands oriented at an angle of $\sim 60^\circ$ relative to lamella. These observations agree with the assumption that the bright bands correspond to dihexadecyl ether molecules, whose central ether oxygens are expected to be slightly darker than the rest of the alkyl chains. Oppositely, bright lines, corresponding to ether oxygens, are clearly observed while imaged with 4MBA modified STM tip (Figure 3b).

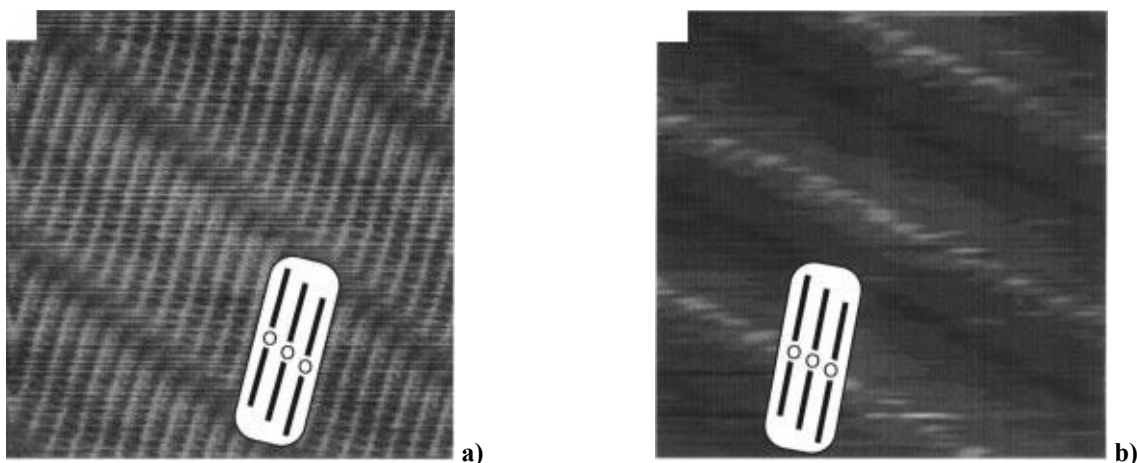


Figure 3. STM images of dihexadecyl ether physisorbed at the 1-phenyloctane/graphite interface, as obtained with (a) an unmodified gold tip (-0.9V, 0.7nA) and (b) a 4-mercaptobenzoic acid modified gold tip (-0.9V, 0.7nA). Image size: 10 nm x 10 nm. [44] – Reproduced by permission of the PCCP Owner Societies.

d. Esters -(C=O)-O-

Finally, this chemical contrast imaging was applied to the identification of ester functional groups, hardly visible otherwise. Self-assembled overlayers of wax esters $[\text{CH}_3-(\text{CH}_2)_{14}-\text{CO}-\text{O}-(\text{CH}_2)_{15}-\text{CH}_3]$, adsorbed at the interface between highly oriented pyrolytic graphite and a solution of phenyloctane, were imaged [39]. Side-to-side packing of lamellae

forms this overlayer. These lamellae, which are separated by troughs appears to be formed by a closed-packing arrangement of parallel sticks, oriented at about 60° with respect to the troughs. However, between each adjacent trough, small depressions are clearly visible when 4MBA modified gold tips are used (Figure 4a). 4-MT modified gold tips also allowed to observe the lamellar structure. However, between each adjacent trough, small mounds are clearly visible (Figure 4b). These images clearly indicate the position of the ester group, which is the only reactive part of the molecule [39, 41, 45].

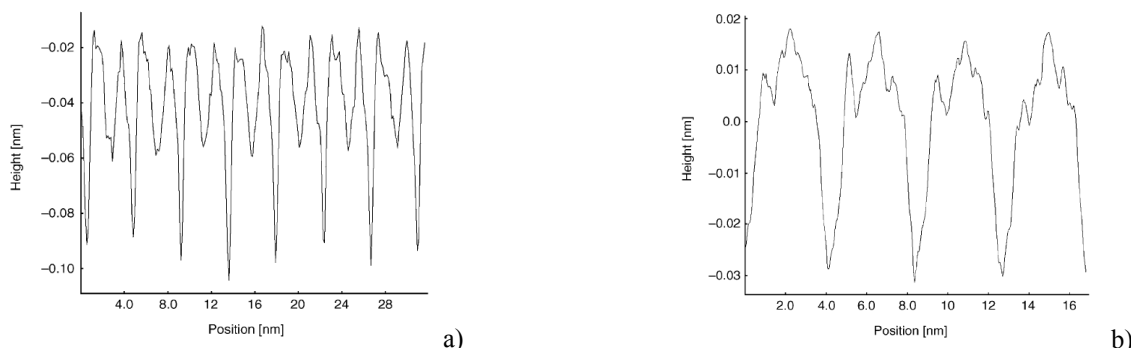


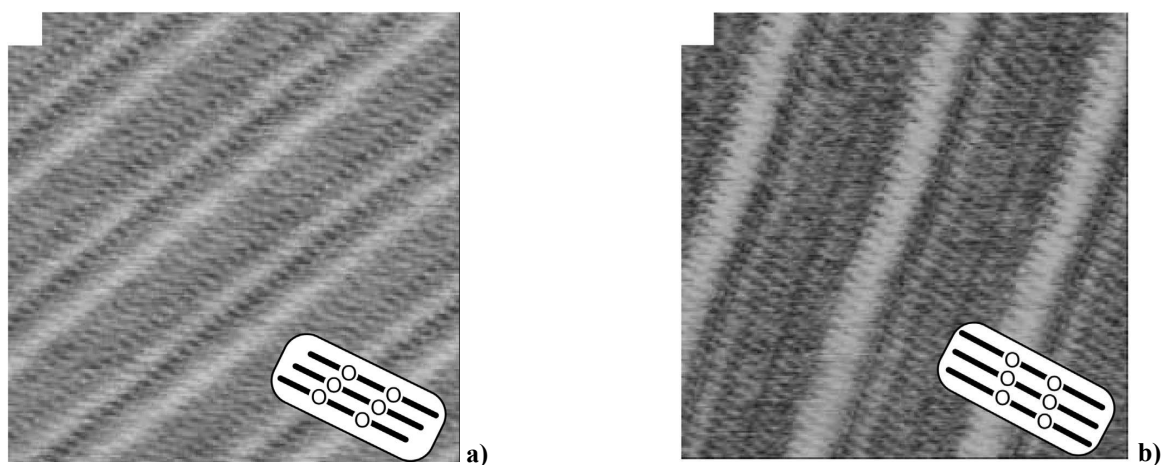
Figure 4. Line profile obtained on STM images of palmitoil palmitate SAM physisorbed at the 1-phenyloctane/graphite interface while imaged with (a) 4MBA modified gold tips (-485 mV, 740 pA) and (b) 4MT modified gold STM tips (-500 mV, 490 pA) [39]. Copyright Wiley-VCH GmbH & Co. Reproduced with permission.

3.2. Chemical groups orientation

Another important information that can be deduced from the chemical sensitive STM imaging technique is the orientation of functional groups on surface. Indeed, the orientation of functional groups able to form hydrogen bond influence the intensity of the hydrogen bond between these groups and the residues on modified tips because different orientations induce a varying extend of orbital overlap. Therefore, it is expected that different orientation of functional groups in SAMs at liquid/solid interface results in different contrast enhancement intensity.

Nishino et al. first demonstrated this assumption by imaging SAMs composed of two different diethers, containing two ether oxygen pointing either in the same ($\text{CH}_3-(\text{CH}_2)_{15}\text{O}(\text{CH}_2)_{11}\text{O}(\text{CH}_2)_{15}\text{CH}_3$, $\text{C}_{16}\text{OC}_{11}\text{OC}_{16}$) or in the opposite ($\text{CH}_3-(\text{CH}_2)_{15}\text{O}(\text{CH}_2)_{10}\text{O}(\text{CH}_2)_{15}\text{CH}_3$, $\text{C}_{16}\text{OC}_{10}\text{OC}_{16}$) direction. They used 4MBA modified gold tips, able to form hydrogen bonds with ether oxygens thanks to their carboxylic groups [43-44].

STM images of both molecules obtained using 4MBA modified gold tips are presented in Figures 5 [43]. In the STM image of SAM composed of the molecules presenting two ether oxygen pointing in the same direction ($\text{C}_{16}\text{OC}_{11}\text{OC}_{16}$), pairs of bright lines are visible (Figure 5a). Based on contrast enhancement and geometric considerations, both bright lines can be assigned to the two ether oxygens included in the diether. Once applied to SAM composed of molecules presenting two ether oxygens oriented in the opposite direction, two bright lines are also observe although exhibiting different intensities (Figure 5b). Following the interpretation stating the enhanced contrast is due to the facilitated electron tunneling through hydrogen bond interaction between ether oxygens on sample surface and carboxyl groups on the tip, both bright lines can also be assigned to ether oxygen position.



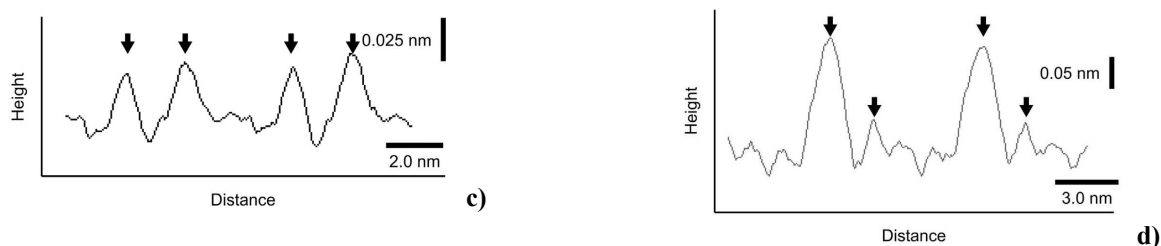


Figure 5. STM image of the $C_{16}OC_{11}OC_{16}$ physisorbed from a 1,2-4trichlorobenzene solution onto HOPG observed with a 4MBA modified tip (a) and an averaged cross-section profile (c) measured perpendicularly to pairs of bright lines. STM image of the $C_{16}OC_{10}OC_{16}$ physisorbed from a 1,2-4trichlorobenzene solution onto HOPG observed with a 4MBA modified tip (b) and an averaged cross-section profile (d) measured perpendicularly to the pair of bright lines. Reprinted from Ref [43], Copyright 2001, with permission from Elsevier.

However, in the $C_{16}OC_{11}OC_{16}$ SAM STM image, the two bright lines are equally enhanced (Figure 5c) although one is much brighter than the other (Figure 5d) in the STM image of $C_{16}OC_{10}OC_{16}$ SAM [43]. These observations allow the deduction of molecule orientation on the substrate. It can be assumed that molecules at the sample surface are adsorbed with their carbon backbone plane perpendicular to HOPG surface. If molecules in the SAM would adsorbed parallel to HOPG surface, the ether oxygen orbitals would point sideways and no difference between relative brightness of both ether oxygen would be observed for any of the studied molecules.

The brightness difference between bright lines in Figure 5b can be related to the orientation of ether oxygen. Indeed, one is pointing towards the solution, while the other one is pointing downwards (towards HOPG surface), the first case being most favourable for hydrogen bond formation than the other one. The difference observed in the brightness of the two ether oxygens is explained by the fact that the ether oxygen pointing towards the solution is forming much stronger hydrogen bond with 4MBA on tip than the other ether oxygen facing the HOPG surface [43]. Similar deductions were also done by Volcke et al. in wax esters SAM [39, 41, 45].

4. Latest developments

4.1. Latest developments in tip functionalization procedures

In order to further improve resolution in chemically sensitive STM images, Nishino and collaborators used carboxyl-terminated single-walled carbon nanotubes (SWNT) immobilized on the apex of STM gold tips. These so-functionalized STM tips provide higher-resolution STM images of diether molecule SAMs at liquid/solid interface [46]. In addition to resolution improvement gained, such tips allowed the selective observation of ether oxygens of sample molecules with high reproducibility, due to the facilitation of electron tunneling through hydrogen bond interactions between ether oxygens in the sample and carboxyl groups at the end of the SWNT tip.

In order to further improve production of modified tip providing a chemical contrast, their functionalization using conducting polymer was tested [47]. They demonstrated that such modified tips could be used to provide molecular resolution STM images. Conductivity of the polymer appears to be an important factor while its layer thickness not. Moreover the chemical sensitive recognition of -OH and -COOH residues, due to hydrogen bond interaction between functional groups on tips and sample, was possible using such polymer coated STM tips [47]. Finally, the reproducibility of the chemical contrast was found to be much higher than for SAMs-modified gold tips. These polymer coated tips appears to be particularly promising for functional groups identification in STM images.

4.2. Latest development in recognized elements

It can be expected that beside hydrogen bond interactions, other types of interactions involving electronic coupling like π - π interactions, metal-coordination or charge-transfer interactions can also be used to specifically identify chemical species in STM images. Further, recently, Ohshiro et al demonstrated a biological sensitive STM imaging by using nucleobase molecular tips to identify biological elements. Precisely, they highlighted the fact that electron tunneling is much more facilitated between a nucleobase sample and its complementary nucleobase molecular tip than with its non-complementary base. This allowed the discrimination of each nucleobase, which was hardly possible otherwise with STM [48]. Similar functionalized STM probes were used by He et al. to perform scanning tunneling spectroscopy measurements in order to „read“ the DNA sequence and yielded a 99.99% accuracy, opening the road to DNA sequencing using H-bond-mediated tunneling as sensor in a nanopore reader [49].

6. Conclusions

In conclusion, the chemical sensitive STM imaging allowed the identification of the STM fingerprint of various chemical functional groups not visible otherwise. Carboxyl, hydroxyl, ether oxygen and esters residues were identified using this techniques. Moreover, this appropriate chemical tip modification technique allows enhanced image resolution and chemical contrast, functional groups orientation determination and in-situ regeneration of the tip properties. It opens the road to structural and chemical identification of molecular structures at liquid/solid interface at the nanometer scale. Further, recent biological sensitive imaging allowing biomolecules (or biologically relevant molecules) to be identified appears to be a very promising derivative of this technique possibly leading in the area of biosensor technology and biological recognition.

Acknowledgements. C. Volcke is a postdoctoral researcher of the belgian fund for scientific research (FRS-F.N.R.S.).

References

- [1] Binnig G, Rohrer H, Gerber Ch, Weibel E. Tunneling through a controllable vacuum gap. *Appl. Phys. Lett.* 1982; 40, 178-180.
- [2] Wiesendanger R. Scanning probe microscopy and spectroscopy: Methods and applications. Cambridge: Cambridge University Press; 1998.
- [3] Martin DS. The adsorption of aromatic acids onto the graphite basal surface. *Surf. Sci.* 2003; 536, 15-23.
- [4] Zhang H-M, Xie Z-X, Mao B-W, Xu X. Self-assembly of normal alkanes on the Au(111) surface. *Chem. Eur. J.* 2004; 10, 1415-1422.
- [5] Dreesen L, Volcke C, Sartenaer Y, Peremans A, Thiry PA, Humbert C, Grugier J, Marchand-brynaert J. Comparative study of decylthiocyanate and decanethiol self-assembled monolayers on gold substrates. *Surf. Sci.* 2006; 600, 4052-4057.
- [6] Pelz JP. Tip related electronic artifacts in scanning tunneling spectroscopy. *Phys. Rev. B* 1991; 43, 6746-6749.
- [7] Binnig G, Rohrer H. Scanning tunneling microscopy. *Helv. Phys. Acta* 1982; 55, 726-735.
- [8] Wiesendanger R, Tarrach G, Bürgler D, Scandella L, Güntherodt H-J. Scanning tunneling microscopy on laser-annealed and thermal-annealed Si(111) – Transitions from 7x7 reconstructed to disordered surface structures. *Ultramicroscopy* 1990; 32, 291-295.
- [9] Israelachvili JN. *Intermolecular and surface forces*. Academic Press, London; 1985.
- [10] Meyer E, Howard L, Overney RM, Heinzelmann H, Frommer J, Güntherodt HJ, Wagner T, Schier H, Roth S. Molecular-resolution images of Langmuir-Blodgett films using atomic force microscopy. *Nature* 1991; 349, 398-400.
- [11] Smith DPE, Horber H, Gerber C, Binnig G. Smectic liquid crystal monolayers on graphite observed by scanning tunneling microscopy. *Science* 1989; 245, 43-45.
- [12] Spong JK, Mizes HA, LaComb LJ Jr, Dovek MM, Fromer JE, Foster JS. Contrast mechanism for resolving organic molecules with tunnelling microscopy. *Nature* 1989; 338, 137-139.
- [13] Clemmer CR, Beebe TB Jr. Graphite: a mimic for DNA and other biomolecules in scanning tunneling microscope studies. *Science* 1991; 251, 640-642.
- [14] Ikai A. STM and AFM of bio/organic molecules and structures. *Surf. Sci. Rep.* 1996; 26, 261-332.
- [15] Culot C, Durant F, Lazarescu S, Thiry PA, Vanloo B, Rosseneu MY, Lins L, Brasseur R. Structural investigation of reconstituted high density lipoproteins by scanning tunnelling microscopy. *Appl. Surf. Sci.* 2004; 230, 151-157.
- [16] Humbert C, Volcke C, Sartenaer Y, Peremans A, Thiry PA, Dreesen L. Molecular conformation and electronic properties of protoporphyrin-IX self-assembled monolayers adsorbed on a Pt(111) surface. *Surf. Sci.* 2006; 600, 3702-3709.
- [17] Morishige K, Takami Y, Yokota Y. Structures of alkanes and alkanols adsorbed on graphite in solution – Comparison with scanning tunneling microscopy images. *Phys. Rev. B* 1993; 48, 8277-8281.
- [18] Claypool CL, Faglioni F, Matzger AJ, Goddard III WA, Lewis NS. Effects of molecular geometry on the STM image contrast of methyl- and bromo-substituted alkanes and alkanols on graphite. *J. Phys. Chem. B* 1999; 103, 9690-9699.
- [19] De Feyter S, Grim PCM, van Esch J, Kellog R, Feringa BL, De Schryver FC. Nontrivial differentiation between two identical functionalities within the same molecule studied by STM. *J. Phys. Chem. B* 1998; 102, 8981-8987.
- [20] Yablon DG, Wintgens D, Flynn GW. Odd/even effect in self-assembly of chiral molecules at the liquid-solid interface: An STM investigation of coadsorbate control of self-assembly. *J. Phys. Chem. B* 2002; 106, 5470-5475.
- [21] DeFeyter S, De Schryver FC. Two-dimensional supramolecular self-assembly probed by scanning tunneling microscopy. *Chem. Soc. Rev.* 2003; 32, 139-150.
- [22] Padowitz DF, Sada DM, Kemer EL, Dougan ML, Xue WA. Molecular tracer dynamics in crystalline organic films at the solid-liquid interface. *J. Phys. Chem. B* 2002; 106, 593-598.
- [23] Wintgens D, Yablon DG, Flynn GW. Packing of HO(CH₂)₁₄COOH and HO(CH₂)₁₅COOH on graphite at the liquid-solid interface observed by scanning tunneling microscopy: Methylene unit direction of self-assembly structures. *J. Phys. Chem. B* 2003; 107, 173-179.
- [24] Stabel A, Heinz R, Rabe JP, Wegner G, de Schryver FC, Corens D, Dehaen W, Suling C. Ostwald ripening of 2-dimensional crystals at the solid-liquid interface. *J. Phys. Chem.* 1995; 99, 505-507.
- [25] Wei G-X, Pan G-B, Wan L-J, Zhao J-C, Bai C-L. In situ STM of phenolic compounds on Cu(111) in solution. *Surf. Sci.* 2002; 520, L625-L632.
- [26] De Feyter S, Gesquiere A, Klapper M, De Schryver FC. Towards two-dimensional supramolecular control of hydrogen-bonded arrays: The case of isophthalic acids. *Nano Lett.* 2003; 3, 1485-1488.

- [27] Bartels L, Meyer G, Rieder K-H. Controlled vertical manipulation of single CO molecules with the scanning tunneling microscope: A route to chemical contrast. *Appl. Phys. Lett.* 1997; 71: 213-215.
- [28] Bartels L, Meyer G, Rieder K-H. The evolution of CO adsorption on Cu(111) as studied with bare and CO-functionalized scanning tunneling tips. *Surf. Sci. Lett.* 1999; 432: L621-L626.
- [29] Lee HJ, Ho W. Single-bond formation and characterization with a scanning tunneling microscope. *Science* 1999; 286, 1719-1722.
- [30] Meyer G, Bartels L, Rieder K-H. Atom manipulation with the STM: nanostructuring, tip functionalization, and femtochemistry. *Comput. Mater. Sci.* 2001; 20, 443-450.
- [31] Bartels L, Rao BV, Liu A. translation and rotation of a haloaromatic thiol. *Chem. Phys. Lett.* 2004; 385, 36-39.
- [32] Ito T, Bühlmann P, Umezawa Y. Scanning tunneling microscopy using chemically modified tips. *Anal. Chem.* 1998; 70, 255-259.
- [33] Volcke C, Thiry PA. Special features in self-assembled monolayer revealed by functionalised STM tips. *J. Phys.: Conf. Series* 2007; 61, 1236-1240.
- [34] Olson JA, Bühlmann P. Scanning tunneling microscopy with chemically modified gold tips: In situ reestablishment of chemical contrast. *Anal. Chem.* 2003; 75, 1089-1093.
- [35] Turro C, Chang CK, Leroi GE, Cukier RI, Nocera DG. Photoinduced electron transfer mediated by a hydrogen-bond interface. *J. Am. Chem. Soc.* 1992, 114, 4013-4015.
- [36] de Rege PJ, Willimas SA, Therien MJ. Direct evaluation of electronic coupling mediated by hydrogen bonds: implications for biological electron transfer. *Science* 1995; 269, 1409-1413.
- [37] Roberts JA, Kirby JP, Nocera DG. Photoinduced electron transfer within a donor-acceptor pair juxtaposed by a salt bridge. *J. Am. Chem. Soc.* 1995; 117, 8051-8052.
- [38] Nishino T, Ito T, Umezawa Y. Selective observation of hydroxy and carboxylate moieties by scanning tunneling microscopy using chemically modified tips with different extents of hydrogen bond acidity or basicity. *J. Electroanal. Chem.* 2003; 550-551, 125-129.
- [39] Volcke C, Simonis P, Durant F, Thiry PA, Lambin P, Culot C, Humbert C. Use of specific functionalised tips with STM: A new identification method of ester groups and their molecular structure in self-assembled overlayers. *Chem. Eur. J.* 2005; 11, 4185-4190.
- [40] Volcke C, Simonis P, Humbert C, Lambin P, Culot C, Thiry PA. On functional groups in STM images of self-assembled monolayers. *Phys. Chem. News* 2007; 36, 34-38.
- [41] Volcke C, Simonis P, Thiry PA, Lambin P, Culot C, Humbert C. Highlighting functional groups in self-assembled overlayers with specific functionalised scanning tunneling microscopy tips. *Nanotechnology* 2005; 16, 2596-2600.
- [42] Hibino M, Sumi A, Hatta I. Molecular motion at domain boundaries in fatty acid monolayers on graphite observed by scanning tunneling microscopy. *Thin Solid Films* 1996; 281, 594-597.
- [43] Nishino T, Bühlmann P, Ito T, Umezawa Y. Scanning tunneling microscopy with chemically modified tips: orientation-sensitive observation of ether oxygens. *Surf. Sci.* 2001; 490, L579-L584.
- [44] Nishino T, Bühlmann P, Ito T, Umezawa Y. Discrimination of functional groups with scanning tunneling microscopy using chemically modified tips: recognition of ether oxygens through hydrogen bond interactions. *Phys. Chem. Chem. Phys.* 2001; 3, 1867-1869.
- [45] Volcke C, Simonis P, Durant F, Thiry PA, Lambin P, Culot C, Humbert C. STM tip functionalisation: A route to chemical contrast imaging. *Physica Magazine* 2005; 27, 415-422.
- [46] Nishino T, Ito T, Umezawa Y. Carbon nanotube scanning tunneling microscopy tips for chemically selective imaging. *Anal. Chem.* 2002; 74, 4275-4278.
- [47] Ito T, Bühlmann P, Umezawa Y. Polypyrrole-modified tips for functional group recognition in scanning tunneling microscopy. *Anal. Chem.* 1999; 71, 1699-1705.
- [48] Ohshiro T, Umezawa Y. Complementary base-pair-facilitated electron tunneling for electrically pinpointing complementary nucleobases. *Proc. Natl. Acad. Sci. USA* 2006; 103, 10-14.
- [49] He J, Lin L, Zhang P, Lindsay S. Identification of DNA basepairing via tunnel-current decay. *Nano Lett.* 2007; 7, 3854-3858.