Scanning probe microscopies for analytical studies at the nanometer scale

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Resum

Les microscòpies locals de rastreig han transformat la manera d'estudiar l'estructura i les propietats d'una gran varietat de sistemes. Sens dubte, han tingut un paper essencial en moltes disciplines, com ara la física, la química, la biologia i l'enginyeria, i han contribuït al naixement de nous camps, com ara la nanociència i la nanotecnologia. El present article intenta destacar la versatilitat i l'alta sensibilitat d'aquestes tècniques per tal de capturar informació analítica a escala nanomètrica. En aquest context, s'intentarà examinar l'evolució d'aquestes tècniques nanoscòpiques des de la seva capacitat per a recollir informació topogràfica, estructura atomicomolecular i processos dinàmics *in situ* fins a determinar localment propietats físiques i químiques. Es presenta una selecció d'estudis il·lustratius basats en aquestes tècniques que abraça diverses àrees de la ciència.

Abstract

The scanning probe microscopies (SPM) have transformed the way of studying the structure and the properties of a wide variety of systems. Without doubt, they have exerted a pivotal role in many scientific disciplines like physics, chemistry, biology and engineering and have helped to give birth to novel fields such as the nanoscience and nanotechnology. This review attempts to highlight the versatility and high sensitivity of these techniques for capturing analytical information at the nanometer scale. In this context we will provide a survey of scanning probe evolution from the capabilities to image topography, atomic/molecular structure and in-situ dynamic processes to the mapping or local probing of physical and chemical properties. A selection of illustrative SPM studies is presented covering several areas of science.

Paraules clau: microscòpia de sonda de rastreig (SPM), microscòpia de força atòmica (AFM), microscòpia d'efecte túnel (STM), espectroscòpia de força, mètodes d'anàlisi local

Keywords: scanning probe microscopies (SPM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), force spectroscopy, local probe-based methods

The advent of the Scanning Probe Microscopies (SPM) has revolutionized the way of approaching and interrogating systems in science, becoming one of the most important breakthroughs in the last 20 years. A more direct exploration of the exciting nanoworld together with its quantum mechanic governing rules became feasible for the first time. Their contribution to surface and material science has been invaluable. They have allowed us to image a wide range of materials, to describe and understand atomically surfaces, interfaces and surface phenomena such as porosity, fractures, defects, grain size, boundaries, and to follow dynamic processes and surface reactions at a molecular level. Polymer science has also profited from the application of SPM techniques; information on uniformity, molecular structure, polymer chains, orientation and dynamics, etc., is now readily achievable. These techniques quickly captured the attention of the bioscience and biomedical community as a long awaited technique for imaging in situ, in real space, real time and with subnanometer scale resolution. Thus, the imaging of biomolecules, the monitoring of biomolecular dynamic events and the sensing of local physicochemical properties became possible. Indeed all these benefits together with the possibility of imaging in physiological conditions, in a non destructive way, with minimal sample preparation and without the requirement of expensive ultrahigh vacuum equipment (as in the case for electron microscopy), make them indispensable tools in the everyday research. As a consequence, an increasing number of SPM studies have unraveled very important issues relating to interesting biomolecules, viruses and cells at the nanoscale. However the capabilities of the SPM techniques have gone even beyond

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that and they have become powerful tools for manipulation of atoms and (bio)molecules and for building functional nanoarchitectures. Thus SPM techniques are playing a pivotal role in all areas of science and constitute an essential resource for the development of nanoscience and nanotechnology.

The main focus of this paper will be on the SPM capabilities for capturing analytical information at the nanoscopic level in material science and biochemistry. The aim is to introduce researchers not experienced with SPM to the profits of these techniques. Thus, the overall purpose of this paper is not to provide an exhaustive review, but rather to give the reader a sense of the ever increasing capabilities of SPM. To that end, we will pick up some illustrative examples from the vast and amazing pool of SPM literature. Although the SPM nanofabrication is a fascinating topic, it will not be covered in this survey. For a more complete and detailed description of the fundamentals, applications and recent developments of the SPM techniques, we refer the interested reader to the following books [1–7] and reviews [8–19].

Basic operating principles

These revolutionary techniques started with the invention of the Scanning Tunneling Microscopy (STM). In this technique a sharp conductive tip is held a few angstroms away from the sample and then moved in an x-y raster scan over a 2D surface (Fig.1a). By applying a voltage between tip and sample, a small current (tunneling current) which flows through the gap is measured. With a negative tip bias, the tunneling electrons originate in filled states of the tip and end in empty states of the sample and the reverse process occurs for positive tip bias. The exquisite lateral and vertical nanometric control is performed by the interplay of two essential parts in the SPM techniques: the x,y,z piezo system and an electronic feedback control. While it slowly scans across the surface, the tip is raised and lowered in order to keep the signal (i.e. the tunneling current) constant with the aid of the servo loop (piezo/feedback system), reproducing the surface topography. The exponential dependence of the tunneling current with the gap distance is the key for the high sensitivity of the STM. Indeed, STM is the most sensitive SPM technique, exhibiting unprecedented lateral and vertical resolution (x,y: 0.1nm, z: 0.01nm) which allows us to obtain atomically re-

solved images of a surface in real space . However, it presents some limitations. Since STM relies on the tunneling current, only conductive or semiconducting surfaces or compounds can be analyzed. Additionally, in many systems, the rational interpretation of STM images is not straightforward and demands a theoretical analysis. The reason is that STM images present maps of partial electron density (electron density at the Fermi level) measured at the tip-sample separation and this partial electron density may not always coincide with the direct position of the atoms themselves. In such cases, a direct correlation between the image and surface topography might be misleading. However, this fact, that can be seen as a disadvantage, is coincidentally very useful since can provide direct information on the spatial location of quantum mechanical orbitals (specific bonding and antibonding orbitals of surface and adsorbed species) and additionally provide chemical contrast that can help to distinguish between and to identify chemically inequivalent atoms.

Atomic Force Microscopy (AFM) was born as a natural desire to extend STM capabilities to nonconducting materials (polymers, biological species, etc.). The principle of AFM relies on the use of a tip mounted on a cantilever which is brought into close proximity to the surface where intermolecular forces acting between tip and sample cause the cantilever to bend (Fig. 1b). The sensing of the forces is by deflection of a light beam, focused on the upper surface of the cantilever which is collected by a positional photodetector. This technique can also achieve very good spatial resolution (x,y: 2–10 nm, z: 0.1nm) and resolve interaction forces with piconewton sensitivity. The first development of the AFM was based on measuring the repulsive forces by contacting the sample with the tip. Since in this standard operating mode the tip is mechanically in contact with the surface, lateral forces are also present and exert a torsional movement of the cantilever as the tip scans the surface. This information can be also collected since the photodetector is a quadrant which measures both normal cantilever bending (due to topographic effects) and torsion. Thus, lateral forces, usually due to differences in friction forces on the sample, can be measured with AFM simultaneously with the topographic images.

However, the dragging motion of the tip in contact AFM, combined with adhesive and lateral forces, can cause substantial damage to soft samples. To alleviate this problem, dynamic AFM modes, in which the topography is monitored by the changes in
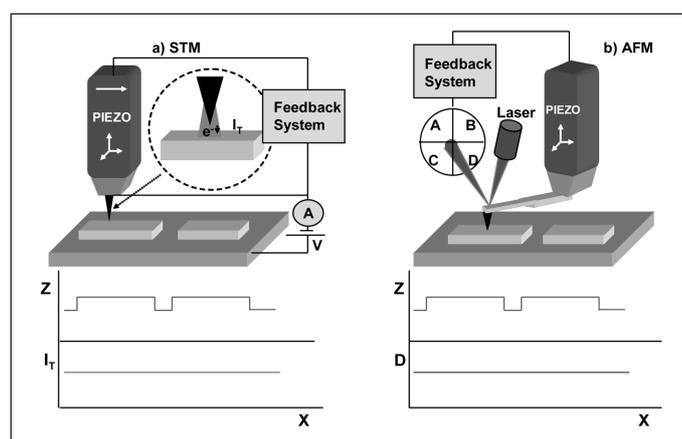

Figure 1. The STM a) and AFM b) setups. In STM the tunneling current between tip/sample is the topographic imaging parameter whereas in AFM is the tip/sample interaction force which is translated into a cantilever deflection signal.

the oscillation of a vibrating tip, were developed (e.g. non contact or intermittent contact AFM mode).Very quickly, it was realized that the dynamic AFM modes could bring about additional valuable information. The existence of several parameters such as the oscillation amplitude, frequency, phase shift and cantilever deflection, which are sensitive to the tip-sample interactions could be used to extract quantitative and qualitative information about material properties at nanometer scale. This sophistication was even increased by applying bias potential between tip and sample and by using AFM tips of a different nature.

One of the aspirations pursued with the SPM techniques is the chemical sensitivity. That is not a straightforward issue. The contrast in SPM originates from the interactions between the tip and the sample. However, these interactions are not only dependent on surface chemistry but also on the morphology, mechanical properties and on the surrounding medium. In order to achieve more direct chemical information, chemically specific forces must be identified and enhanced and the other forces reduced or eliminated. One way to accomplish that is by the specific chemical modification of the SPM tips, a procedure that brought about the Chemical Force Microscopy. This technique can be used in combination with the lateral force and phase mode, and with force spectroscopy and will be discussed later.

As can be observed, STM and AFM became the platform for an emerging wealth of related techniques with the capability of providing not only topographical information but also property mapping or more local property information. Additionally, these techniques can be used in different environments (air, electrochemical, ultra high vacuum). The evolution from a qualitative imaging tool to a quantitative probe of interaction forces and material properties was readily seen.

Table 1 shows some examples of such techniques with their corresponding obtained information.

Table 1. Outline of some of the most widely used SPM techniques.

| SPM mode	Measured Parameter and acquired information	
STM	Tunneling current	Topography Spatial distribution of local electronic states (LDOS)
Contact AFM	Vertical cantilever deflection *Repulsive forces*	Topography
Intermittent AFM (oscillating tip)	Changes in the tip oscillation amplitude *attractive/repulsive forces*	
Non-contact AFM (oscillating tip)	Changes in the tip oscillation amplitude or frequency *Attractive forces*	
Lateral Force Mode (contact)	Lateral cantilever deflection *Friction Forces* *Surface Composition (differences in chemical, mechanical properties, etc.)*	Mapping of Chemical and Physical Properties (mechanical, electronic, magnetic properties, etc.)
Phase Mode (oscillating tip, intermittent contact)	Phase shift *attractive/repulsive forces* *Surface composition, differences in local mechanical (elasticity, stiffness), adhesive, chemical properties*	
Force Modulation Mode (oscillating tip but all the time in contact)	Changes in the vertical averaged cantilever deflection *Mechanical Properties (elasticity, stiffness, etc.)*	
Electrostatic Force Mode (oscillating tip, intermittent contact)	Phase shift *Electrostatic forces* *Charge density, electric field gradient*	
Current sensor AFM (contact mode)	Current *conductivity mapping*	
Magnetic Force Mode (oscillating tip, non- contact)	Phase shift *Magnetic forces, Magnetic force gradient*	
Thermal Scanning Mode	Temperature *Thermal conductivity distribution*	
Force Spectroscopy (static or oscillating tip)	Cantilever deflection vs. Z displacement. Also phase and amplitude vs. Z can be acquired (vibrating tip). *Adhesion, binding forces and mechanical properties*	Local probing of properties
Tunneling Current Spectroscopy	Tunneling current vs. Voltage or vs. Z displacement *Conductivity, LDOS, material work functions, chemical sensitivity*	
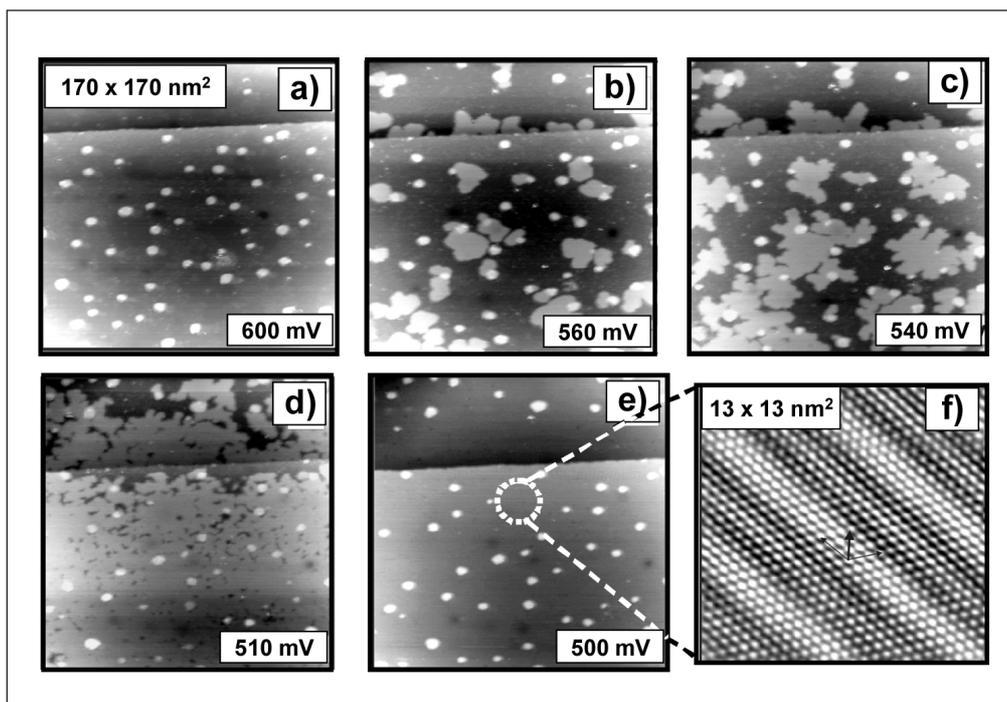
Figure 2. Monitoring of the silver underpotential deposition on single Au crystal (Au(111)). a) shows the bare gold substrate together with some gold islands and a gold step, b), c) and d) show the stages of the Ag adlayer growth from the gold defects (islands, steps) to cover all the surface reproducing the lying topography (e). A high resolution STM image of the Ag layer atomic structure forming a stripped pattern can be observed in f). Images adapted from Ref. [22].

One could also mention other closely related techniques like the Scanning Electrochemical Microscopy (SECM). The overall instrumentation is similar to that used in STM in an electrochemical environment. However the principles of operation and the type of information obtained differ from those of STM. SECM provides chemical information based in the diffusion of redox active species between a nanoelectrode (tip) and the sample. SECM turns out to be very useful for mapping zones of varying chemical and electrochemical activity at micron/submicron resolution, for measuring reaction kinetics, ion fluxes through membranes, localized ion selective sensing, etc. This technique can be considered as an extension of the classical electroanalytical methods at the submicron level. Additionally, the techniques based on optical fibers such as the scanning near field optical microscopy (SNOM) or near field scanning microscopy (NSOM) can be included in this extended SPM family. For more information about SECM and SNOM techniques, the reader is referred to the literature [20,21].

In the next section we will introduce some illustrative examples of how some of these techniques can be used in order to get not only invaluable topographical information, but also material property information in a wide variety of disciplines.

STM applications

Topography and imaging of dynamic processes

With the use of well defined surfaces as single crystals, STM has helped to answer many questions related to surface processes, such as surface reconstruction, surface adsorption and deposition, catalysis, corrosion and oxidation processes, self-assembled structures, etc., and has given clues for mechanistic interpretation. In the literature one can find several examples of how STM provides insights into the balance between intermolecular interactions, molecule-substrate interactions and thermal energies that govern diffusion, nucleation and self-organization of molecules on surfaces under different environments, ultra high vacuum or electrochemical medium. As an example, Figure 2 shows the STM monitoring of a surface process under electrochemical environment: the electrodeposition of a silver adlayer on a gold electrode as the potential is negatively scanned [22]. At 500 mV the Ag layer has grown on the overall surface reproducing the starting substrate topography. At such a potential the silver atom rearrangement on the gold surface can be closer inspected.

An important group of ordered adlayers, which has been intensively studied by STM, are the self-assembled monolayers (SAMs). These are formed by molecules with a functional group that interacts strongly with the substrate. A classical system is alkanethiols on gold, particularly on Au(111), where the strong interaction between gold and sulfur leads to a stable and ordered adlayer upon mere immersion of the gold substrate into a thiol solution. STM has contributed significantly to the understanding of the monolayer properties by yielding structural information in different media. The structure of the organic monolayers clearly depends on the terminal groups and the chain length which modify the intermolecular as well as the adsorbate-substrate interaction (Figure 3, electrochemical environment) [23]. Striped phases of lower molecular density are frequently observed for short chain thiols. A closed packed hexagonal layer was observed by longer methyl terminated alkanethiols. Normally, the electron density of the thiol or oxygen atoms are the responsible of the topographic contrast (brighter spots) whereas the local density of electronic states of
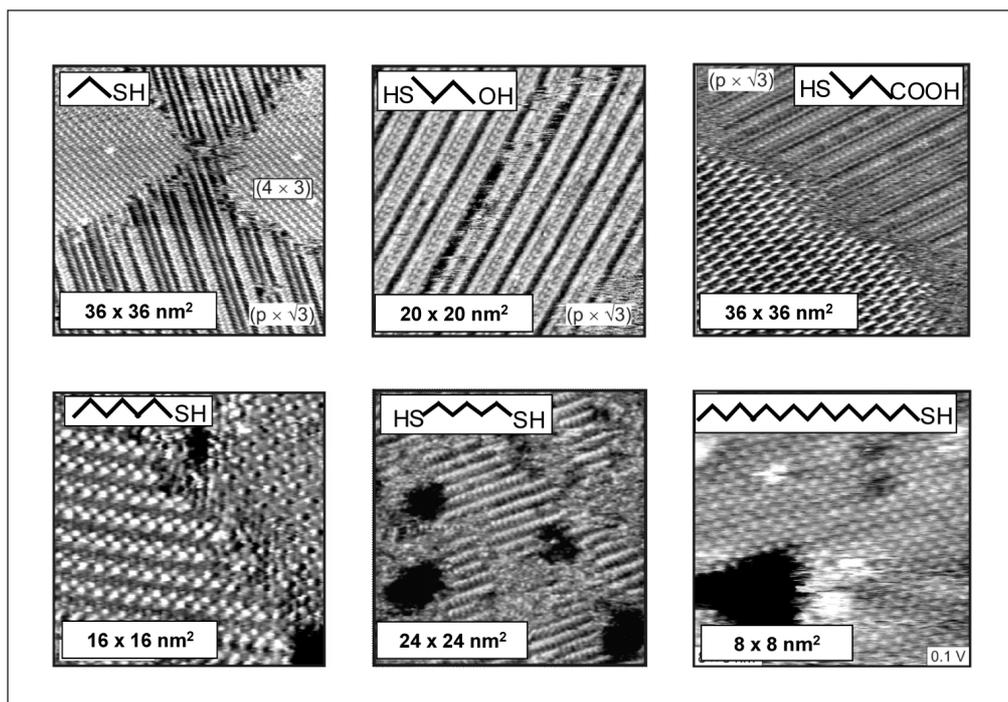
Figure 3. In-situ STM image of different chain length and functional end-group SAMs in an electrolyte under a potential at which the SAM is stable. Note the different SAM patterns. The thiol molecule rearrangement is very dependent on the chain length and on the terminal functional group. The images have been adapted from Ref. [23].

the –CH_2 or CH_3 can not be easily spatially resolved under these conditions.

Mapping or local analysis of material properties

In many systems, STM images are strongly dependent on the tip/sample voltage. The collection of STM images at different tip/sample voltages is one simple way to acquire spectroscopic information. Spectroscopic information can also be obtained by positioning the tip over a selected location and measuring the current vs. potential profiles. To speed up and improve the collection of spectroscopic information, some research groups developed the Scanning Tunneling Spectroscopy (STS) [1,3]. In the scanning mode, the applied bias is modulated as it is slowly swept keeping the tip position essentially constant while the variation in the tunneling current is measured. One can obtain the differential tunneling conductance which is related to the density of states in the sample and enables one to distinguish regions of the surface of different chemical nature, to detect the presence of adsorbed layers and so on. One can also perform tunneling spectroscopy by varying the gap distance at a constant applied voltage. This technique can also be employed either at a fixed x and y position of the sample or with scanning. Thus, one can get information on the local height potential barrier for electrons (local work function) or on the spatial distribution of work functions of the surface when the tip is scanned.

Figure 4 shows the constant current STM image and the differential conductance dI/dV maps for a system consistent on Co islands on Cu (111) substrate at low temperature [24]. The differential conductance is proportional to the local density of electronic states (LDOS). On the Cu(111) surface , we observe the well known standing wave patterns in the LDOS which are due to quantum interference of surface state electrons scatter-

ing off steps and defects. A similar oscillation pattern is observed on the Co islands, evidence of a free electron like surface state.

Figure 5 shows an example of how tunneling spectroscopy enables the characterization of the electronic properties of carbon nanotubes [25]. Specifically, current vs. voltage was measured at specific sites along the tubes and differentiated to yield the normalized conductance (V/I)dI/dV, which provides the LDOS. The first case corresponds to a metallic nanotube
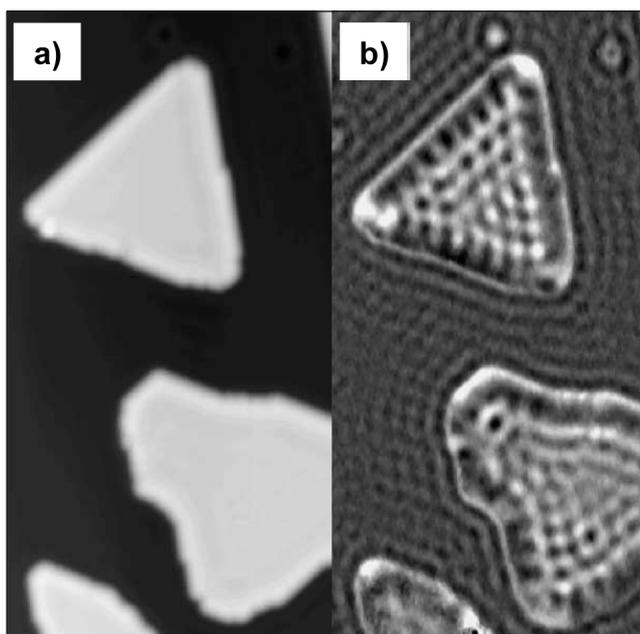

Figure 4. A typical STM image of triangular Co islands grown on Cu(111) at low coverage (a). In b) the simultaneous dI/dV maps are obtained. Brighter areas correspond to higher topography and larger DOS respectively. Images adapted from Ref. [24].
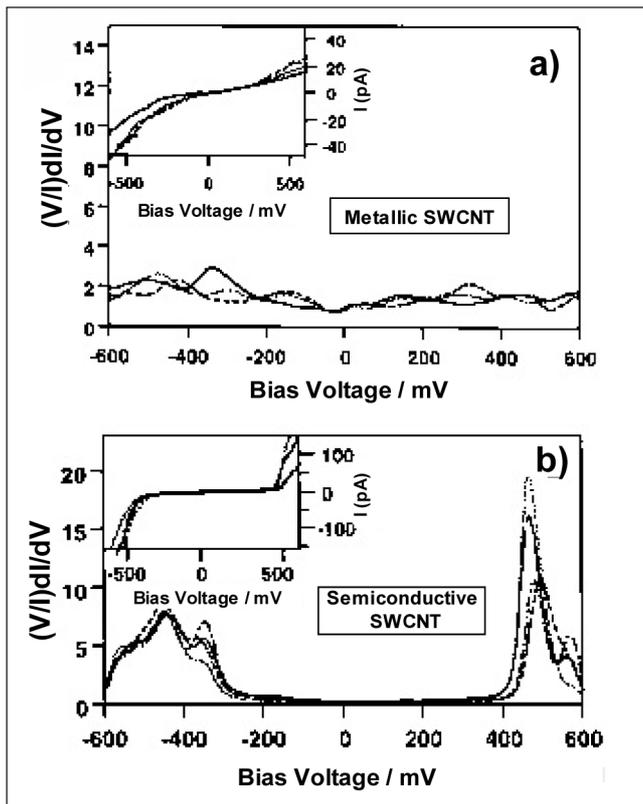
Figure 5. Typical current/potential curves and correspondent normalized conductance with LDOS information for a metallic (a) and semiconducting (b) nanotube respectively. The different curves in (a) and (b) correspond to distinct locations on the nanotube. Curves adapted from Ref. [25].

with almost constant LDOS. The second case corresponds to semiconducting nanotubes. Their I/V curves are consistent with a semiconductor material and the normalized conductance shows sharp increases at positive and negative bias which correspond to the conduction and valence band edges in the LDOS.

Finally and only to briefly mention, STM operated at low tem-
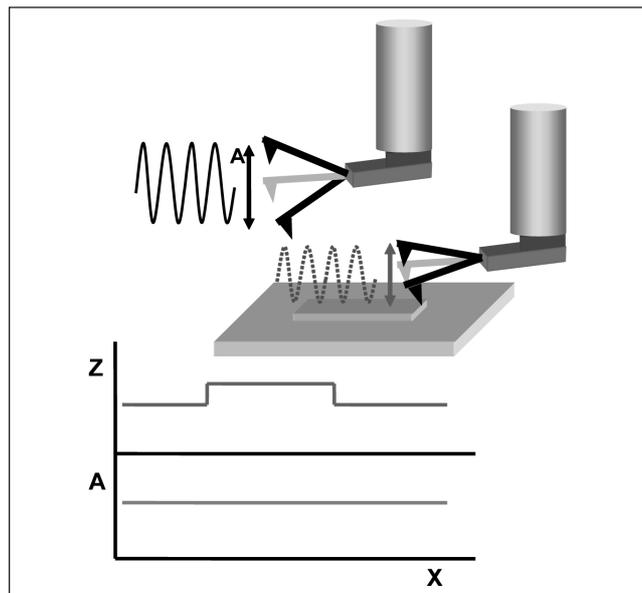

Figure 6. Scheme of the intermittent contact mode imaging principle. As soon as the oscillating tip approaches the surface, the interaction forces decrease the oscillating amplitude and this signal is used for tracking the substrate topography.

peratures has become a powerful tool not only to probe and manipulate single atoms and molecules but also to measure vibrational spectra of molecules via inelastic tunneling processes which excite their vibrational modes [26]. The changes in electron conductance appearing as peaks in plots of d^2I/dV^2 vs. V can be correlated to vibrational excitations. Such studies involve the rotation of single molecules, bond selected chemistry, dissociation or bimolecular reactions, the measurement of vibrationally resolved fluorescence spectra from different parts of individual molecules (e.g. porphyrin), etc. The high spatial resolution of the STM permitted the researchers to map out the intramolecular features of the molecular photon emission process [26].
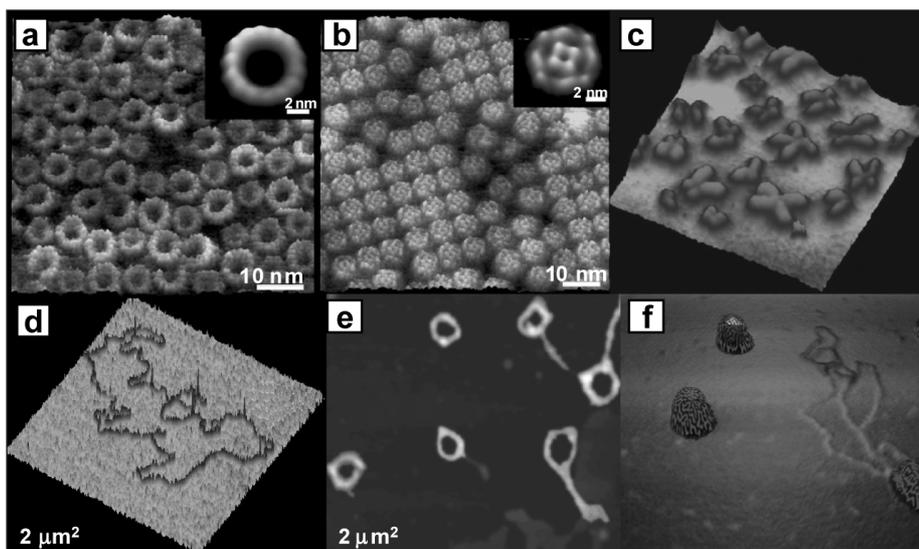

Figure 7. Collection of high resolution AFM images of different biological systems. The first image (a) is an AFM image of the proton driven rotor (F_0 rotors) from chloroplast ATP synthase (image adapted from Ref. [27]). This technique has helped to unravel the structure of such rotors. A closer look allows seeing the 14 subunits conforming the rotor. Figure 7 b) shows the topography of a vesicle densely packed with Aquaporin Z tetramers exposing their extracellular surface (image adapted from Ref. [27]). A closer look shows better the crown like appearance and the twelve surface protrusions per tetramer. Fig. 7 c shows a high resolution image of chromosomes (Ref. [28]). Fig. 7 d shows a mutated endonuclease (light gray spikes) as a marker to tag a specific sequence of DNA (image adapted from Ref. [29]). The images are clear and support the application of AFM imaging to map the physical locations of certain enzymatically active sites. In e) the condensation of DNA can be visualized (Ref. [30]) whereas in f) a three– Dimensional AFM image of disrupted and intact adenovirus on native silicon oxide can be seen. DNA is uncoiling out from one viral capsid (image adapted from Ref. [31]).

Applications of AFM and related techniques

Topography and imaging of dynamical processes

From the three AFM topographic modes, the intermittent contact one has become one of the most used for imaging soft materials since it combines good resolution together with lower degree of sample damage (friction and lateral forces are minimized) [9]. This intermittent contact AFM mode is accomplished by first oscillating the cantilever at or near its resonant frequency. The oscillating tip is then brought towards the surface until it begins to slightly touch or tap the surface, which reduces its oscillation amplitude. The reduction in the oscillation amplitude now becomes the feedback control signal which can be used to follow the surface topography (Fig. 6). Biology has collected invaluable benefits from this technique which has allowed the investigation of the structure and dynamics of many biological systems (proteins, polysaccharides, polynucleotides, lipids, membranes, antibody/antigens, viruses, living cells in their native state, etc). Among the biological processes, many efforts have been focused on the DNA research field, studying problems such as DNA interactions with proteins, lipids and drugs. Direct imaging with AFM has been used to identify specific nucleotide sequences in plasmid DNA molecules via interaction with mutant restriction enzymes. Transcriptional activity of RNA polymerase and DNA condensation and repair processes, antibody binding to surface antigens in membranes, RNA/DNA release from viruses, conformational changes of membrane proteins and biopolymer growth have also been investigated [10,12,19,27,29–32].

Figure 8 depicts an example of a dynamic follow up of a biological process. Specifically, it illustrates how the time-lapse AFM can be used to monitor the growth of amyloid fibril in vitro, a process that occurs in many diseases [32]. With this technique it was possible to visualize the nucleation, elongation, branching, or lateral association of protofibrils and also give hints to implementing therapeutic actions.

Mapping of physical and chemical properties

Lateral force microscopy (LFM)

As previously mentioned, in this technique the torsion or twisting of the cantilever due to the different frictional forces is recorded as the tip is scanning the sample in contact mode. This technique allows the identification and mapping of relative differences in
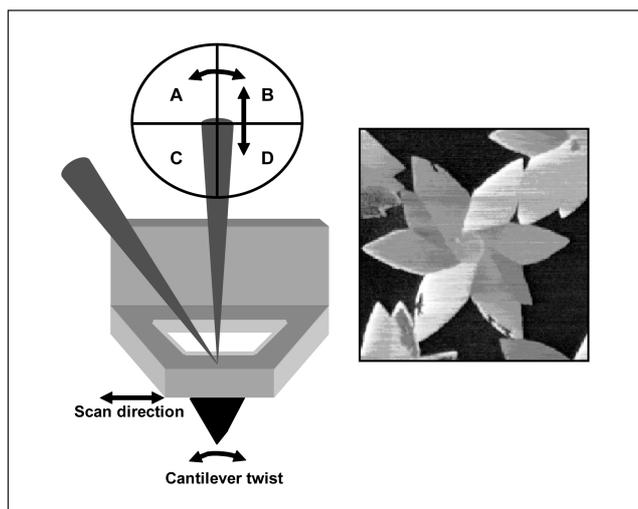
Figure 9. Lateral force mode scheme. Friction image of a lipid monolayer on mica (16×16 μm^2). The image revealed flower-like condensed domains due to friction anisotropies induced by the molecular tilt. Image adapted from Ref. [33].

surface frictional characteristics providing information especially on local variations in chemical composition. Thus, it has been proved to be particularly useful for differentiating components of a heterogeneous surface and it has been successfully applied to polymer science, to detect and delineate different coverage surface layers. Fig. 9 shows an example of how the LFM can be used to capture molecular orientation of a lipid monolayer on mica, impossible to be detected by only monitoring the topography [33]. The monolayer exhibits condensed domains with different molecular tilts which cause different contributions to friction.

As already mentioned, one of the limitations of the SPM techniques is the lack of direct chemical sensitivity. However a more selective approach to study chemical properties can be achieved by functionalizing the AFM probe to sense a specific chemical or biological interaction. This technique, which is named Chemical Force Microscopy [34], can be used either in a local way by performing force spectroscopy (see below) or by performing surface mappings. Thus, by utilizing chemically functionalized tips, force microscopy can be used to probe forces between different molecular groups, measure surface energetics at a nanometer scale, determine surface pK values due to acid and base terminal groups, map spatial distribution of specific functional groups and their ionization state, etc. One model system that evidences the chemical contrast under the
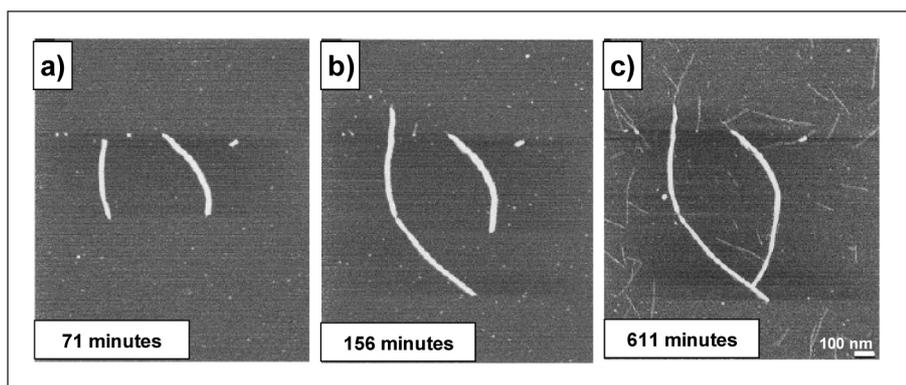
Figure 8. Nucleation and growth of amylin protofibrils on mica. Images adapted from Ref. [32].
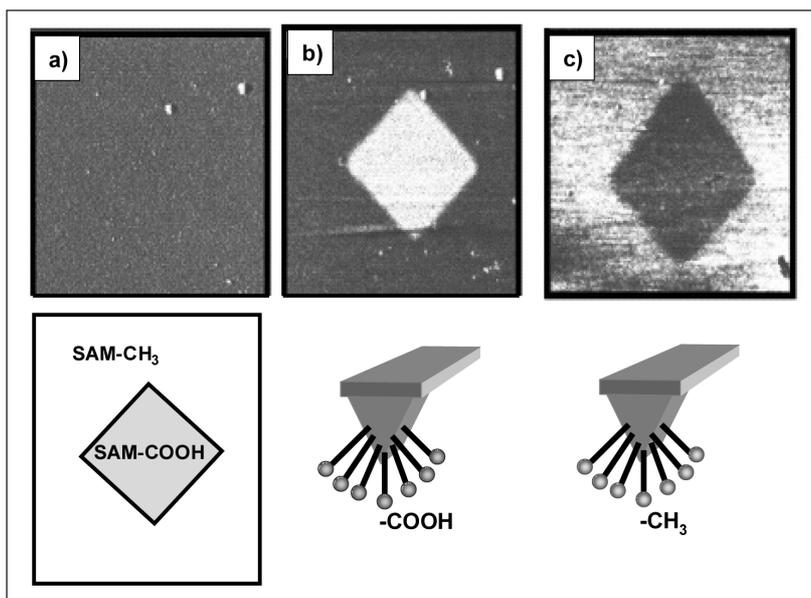
Figure 10. Force microscopy image of a photopatterned SAM sample in ethanol. The scheme below shows a region of the surface patterned with -COOH terminated alkanethiols (inner square) and the surroundings correspond to -CH$_3$ terminated alkanethiol layer. In the topographic image (a) the pattern could not be visualized. However the friction images (b and c) show the patterned area. In both friction images, dark regions indicate low friction whereas light regions indicate high friction. In b) the tip was functionalized with -COOH terminated SAM. Higher friction forces were seen when the tip was scanning the -COOH terminated SAM region of the substrate due to the increase of the hydrogen bond interactions. Images recorded with methyl terminated tips exhibit a reverse contrast. Higher friction is observed in the -CH$_3$ terminated surface regions (due to the hydrophobic forces between the methyl groups). The AFM Images have been reproduced from Ref. [35] with permission of the American Chemical Society.

lateral force mode with chemical modification of the probe tip is depicted in Fig. 10 [35]. Photochemically patterned self-assembled monolayers (SAMs) of alkanethiol on Au with different terminal groups (-CH$_3$ or -COOH) are depicted. Topographical images failed to reveal the pattern since such surfaces exhibit almost flat topography. Friction images of these samples taken with different functionalized tips show chemical information about the surfaces.

AFM has also been used to probe highly specific ligand/receptor complexes such as antibody-antigen interactions, avidin-biotin, complementary DNA strands, etc., in order to generate molecular maps in a compositionally complex sample with simultaneously high spatial resolution [11].

Phase Mode

Previously we have seen that intermittent contact mode is characterized by a sinusoidal oscillation with particular amplitude that is imposed to the probe by an external driving force. Such amplitude is the set point amplitude which is taken as the parameter for the feedback and for generating the topographic image. In this mode, the phase lag between the tip oscillation and the excitation signal can be also captured and it provides additional information [9]. The phase shift is related to tip-surface interaction which is basically material specific and it can thus be used to map functional group distributions. Numerous papers have investigated the relation between the phase lag and the variations in material composition and properties such as adhesion, stiffness/elasticity and viscoelasticity [9]. The intermittent contact mode and particularly the phase measurements have been extensively used for polymer research [7]. As examples of these applications, we can mention the direct visualization of the polymer chains, the nanometer scale architecture of crystalline polymers, block copolymers, three dimensional compositional maps of heterogeneous polymer systems and polymer thermal phase transitions, etc.

Here we will show some examples of the contrast obtained by phase images. The crystallization in block copolymer me-
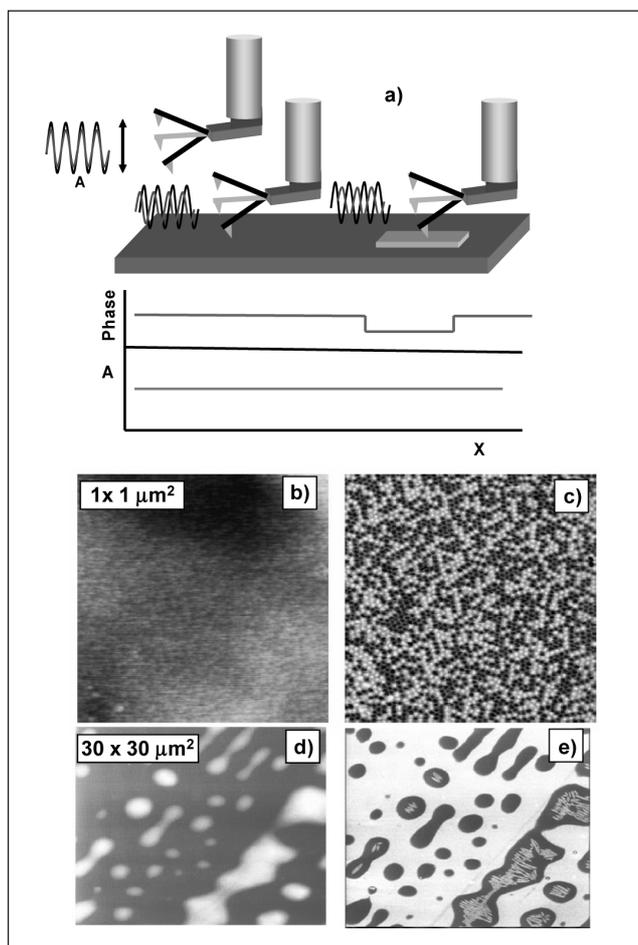

Figure 11. Illustrative scheme of the phase imaging mode (a). A phase lag in the oscillating tip is induced by the interaction forces with the substrate. The phase lag magnitude is dependent on the material nature. b) and c) correspond to the AFM topography and phase image respectively of a thin diblock copolymer (PB-PEO) after partial crystallization at −25 °C, adapted from Ref. [36]. The dark disks visible in the phase images represent molten PEO micelles whereas the lighter ones represent crystalline PEO micelles which are much stiffer. The PB forms the continuous matrix at where the PEO organizes. AFM images of PDES on Si, d) topography and e) phase image are also depicted (images adapted from Ref. [7]).
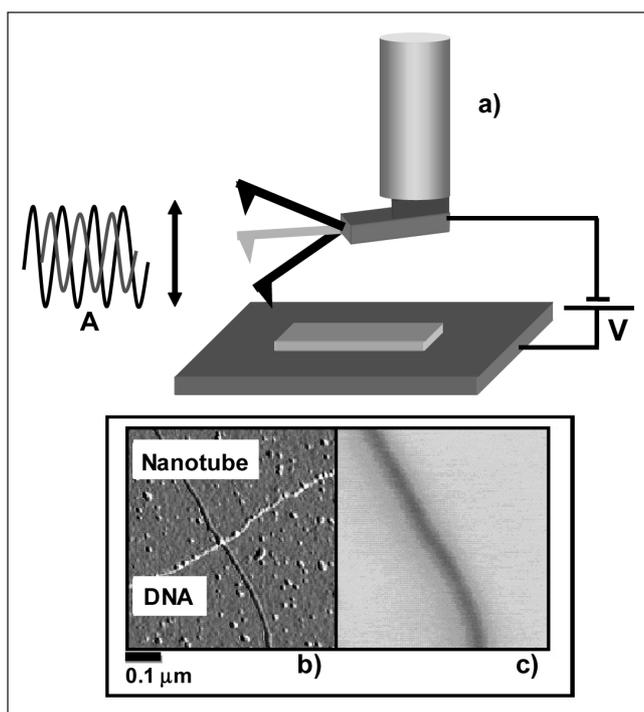
Figure 12. EFM scheme (a), topographic (b) and EFM (c) images of an insulating substrate containing both a single walled carbon nanotube and a λ-DNA strand. DNA is not detected by phase contrast in the EFM image, indicated that the measured DNA is an insulator, although both were equally visible in the topographic image. Images adapted from Ref. [37].

sophases (poly(butadiene-b-ethyleneoxide, PB-PEO) is shown in Fig. 11 b and c [36]. Phase imaging proved to be an efficient tool to resolve differences in the viscoelastic properties of polymers and its organization in a spherical mesophase for PEO.

Another example of how to unravel heterogeneity with the phase imaging is also presented in Fig 11, which shows the height and phase images of several poly(diethylsiloxane) (PDES) patches, deposited on Si substrate by rubbing (d and e) [7]. The height image shows striated droplets of polymer on the Si. The phase contrast indicates three different materials present on the surface. The brightest areas correspond to the Si surface whereas the darker spots correspond to the amorphous PDES. Inside the polymer droplets brighter lamellar aggregates are seen which are not distinguishable in the topographic image.

Electrostatic Force Microscopy (EFM)

Electrostatic Force Microscopy is a powerful tool to image electric field gradients. For example, amongst its many diverse applications, it has been employed to image potentials in carbon nanotube circuits, failure check on integrated circuits and to study ferroelectricity. The EFM is based on a vibrating conductive tip which is biased with respect to the sample. In order to decouple the electrostatic interactions from the surface topography, a two pass lift technique must be used. In this mode the topography is measured in the first scan and the electric properties are measured in the second scan. In this second scan, the probe motion reproduces the measured surface profile (feedback is off) but with a vertical lift of 20–50 nm. Changes in phase of the oscillating probe during the second scan reveal surface variations in the electric field. This minimizes cross-talk

with topographic data. A very interesting application has been reported by Bockrath et al. [37]. They devised a scanned probe technique based on EFM capable of probing the conductance of samples without requiring attached leads. This technique has been successfully used to give hints in an intense debate on whether the DNA is conductive or not. In contrast to conducting single walled carbon nanotubes, used as control, individual strands of λ-DNA, a widely studied sequence, are found to be insulating on the DNA length probed.

Current sensor mode

This technique consists in the use of a conductive tip operating in standard contact mode. As a voltage is applied between tip and sample, a current is generated, the intensity of which will depend on the sample nature. Thus, a map of current distribution along the sample is measured simultaneously with the surface topography. In contrast to STM, the topographic information in current sensor mode is substantially decoupled from the local electrical conductance properties, thus allowing a reliable simultaneous measurement of both properties. Additionally, current sensor mode is most suitable for samples with low conductance or composite materials containing insulating regions where an STM would lose its feedback signal. An illustrative example is depicted in Fig. 13 which shows the topography and conductivity images of a carbon epoxy composite material composed of cylindrical carbon fibers embedded in dried epoxy resin. The conductivity data show zero current above the insulating epoxy regions and current above the conductive carbon fibers [38].
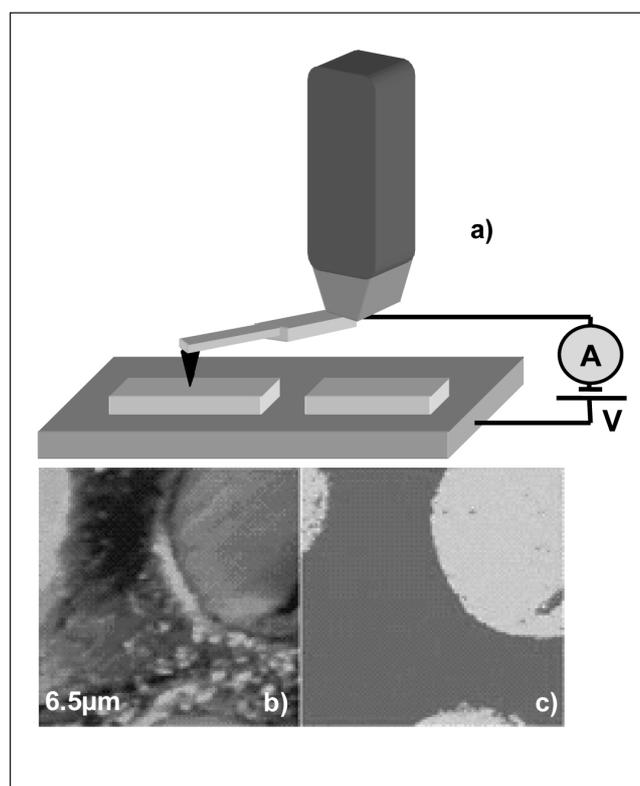

Figure 13. Current sensor mode scheme (a). Topography (b) and conductivity (c) image of a carbon fiber embedded in a resin matrix. Lighter regions correspond to higher conductivities. Image adapted from Ref. [38].
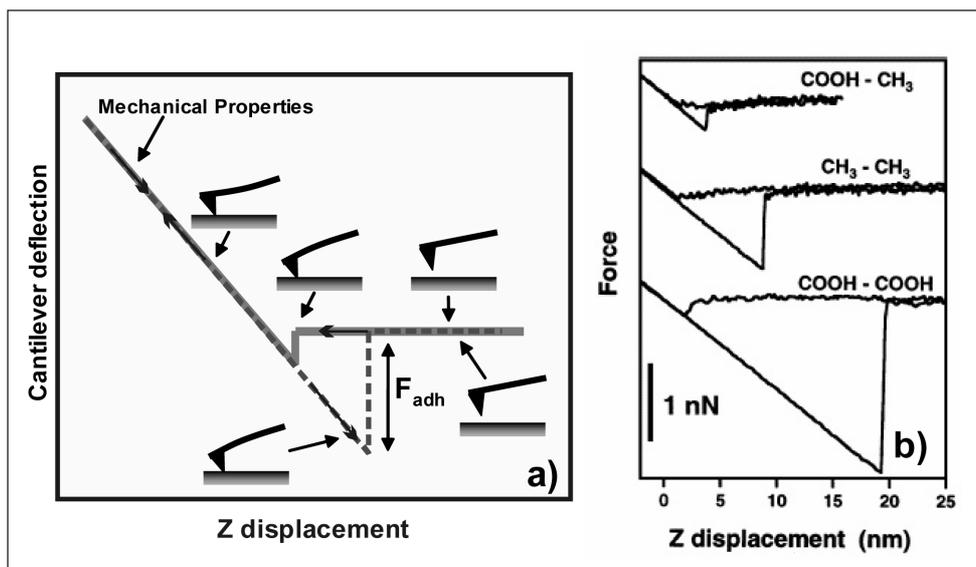
Figure 14. Typical force vs. tip Z displacement curve (a). Representative force-displacement curves in ethanol for COOH/COOH, CH_3/ CH_3 and CH_3/COOH tip/sample functionalization (b). These curves have been reproduced from Ref. [35] with permission of the American Chemical Society.

Local probing of physical and chemical properties

Force Spectroscopy

Force spectroscopy constitutes a non imaging application of the AFM which allows a more quantitative analysis of the interaction forces. It also provides quantitative measurements of different physical/chemical properties. Figure 14 a) shows a typical force vs z displacement curve. As the probe approaches the sample, the tip initially experiences a small attractive force which causes the cantilever to bend downwards. At short distances, the Van der Waals attraction will pull the tip abruptly into contact with the sample. After that, the cantilever deflects upwards, showing the repulsive tip-sample forces. This part of the curve profile provides hints as to the mechanical properties such as the material stiffness/elasticity. When the tip is retracted hysteresis can appear since the tip may be stuck to the sample by adhesion or capillary forces (very common in air medium). The maximum force necessary to pull the tip off the sample surface corresponds to such adhesive or capillary forces. This methodology has been used to rationally probe interactions between surface functional groups with chemically modified probes (hydrophilic, hydrophobic, charge interactions). If capillary forces are not desirable for force interaction interpretation, ambient control must be used, either working in vacuum or in inert or liquid medium. As previously mentioned, the analysis can be extended to the quantification of surface free energies, determination of local pKs of surface ionizable functional acid/basic groups, determination of the binding forces between ligand/receptors, etc., [34]. Figure 14 b) shows force/displacement curves for tips derivatized with either -CH_3 or -COOH terminated SAMs and samples also modified with -CH_3 or -COOH terminated SAMs. The experiments, carried out in ethanol, probed the different adhesion forces between the interacting groups. The observed trend in the magnitudes of the adhesive interactions between tip/sample functional groups, that is COOH/COOH > CH_3/ CH_3 > CH_3/COOH, agrees with the qualitative expectation that the interactions be-

tween hydrogen-bonding groups (COOH) will be greater than between non-hydrogen bonding groups (CH_3).

One striking contribution in this area has come with the single molecule force spectroscopy (SMFS). This has become a very exciting area allowing the investigation of inter/intramolecular forces of biomolecules at the single molecule level with a sensitivity in the range of piconewtons [39,40,41]. These intra/intermolecular forces are the responsible for the different molecular conformation and structures. Therefore the analysis of molecular stretching, folding/unfolding mechanisms, the binding forces, etc. provides insights for the molecular structure, which, in turn, facilitates the understanding of its function. In this context, many works have used force curves to probe interactions in single proteins, polysaccharides, between complementary DNA strands, biotin/avidin, antigen/antibody, drug/enzyme complexes, etc., [39–43].

In the latter examples, the force experiments consist of attaching a ligand to the tip (e.g. avidin, DNA strand, etc.) and then approach the sample which is functionalized with the corresponding receptor (biotin, complementary DNA strand, etc). These experiments have allowed monitoring of the entropic forces, bond torsions, conformational and structural transitions and bond ruptures [39–43]. The main applications of force spectroscopy have been focused on unraveling mechanical issues in different proteins (titin, fibronectin, ubiquitin, membrane proteins) and on DNA [39–43]. The forced extension of an individual double strand DNA (dsDNA) molecule gives a force/extension curve with characteristic features [39,42,43]. The mechanics of DNA overstretching is sequence dependent and force spectroscopy can be used to measure directly the base pairing forces of C-G and A-T nucleotides [43]. Additionally, the interactions of dsDNA with other molecules (e.g. protein, drugs) influence its force spectra, with differences that are characteristic of the particular molecule-DNA interactions [39,42,43]. This indicates that AFM based force spectroscopy is a very sensitive tool to study basic mechanisms of biological importance.
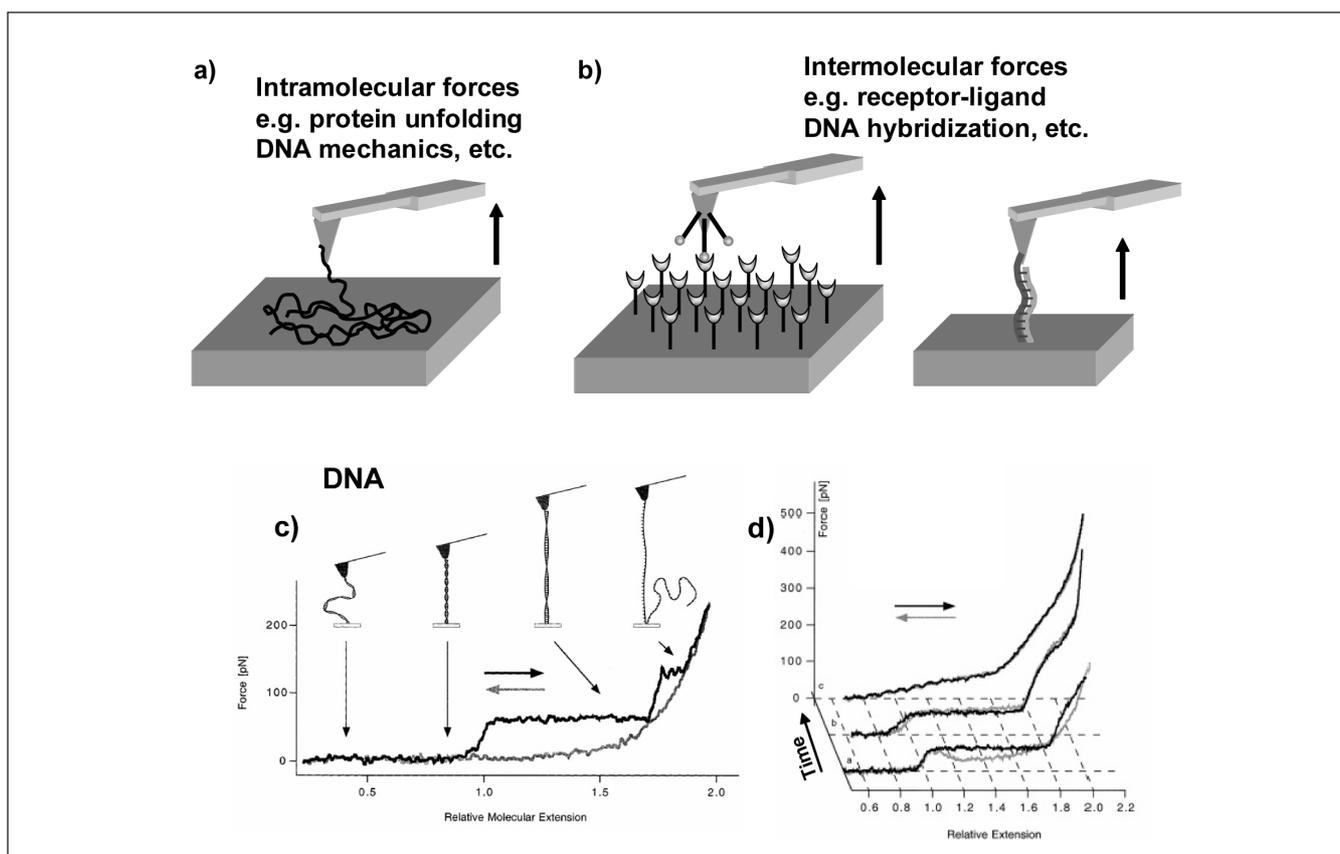
Figure 15. a) and b) illustrative schemes of how force spectroscopy can be used to probe intramolecular and intermolecular forces. c) Schematic of the force extension characteristics of DNA up to splitting into two single strands. d) Effect of adding a drug (cisplatin) on the DNA characteristics force extension curve. The progress of the chemical reaction is followed on the same molecule. Notice the changes in the force extension profiles and how the hysteresis between stretching and relaxation is vanished. The curves have been reproduced from Ref. [42] with permission of the Federation of the European Biochemical Societies.

The use of carbon nanotubes as AFM probes

The unique properties of single walled carbon nanotubes have determined their viable use as research tools in the last years. Very quickly it was realized that the conjunction of their electronic properties, mechanical stability, small diameter/ high aspect ratio and well defined surface, make them very suitable as probes for scanning nanoscopies. With such capabilities, the enhancement of high resolution topographic imaging together with the less aggressive interaction between tip/sample has now been achieved. The pioneer works of Lieber et al. showed that it was possible to attach multi (MWNT) or single (SWNT) walled-carbon nanotubes to conventional silicon AFM probes. These nanotube tips have proved to be high resolution imaging probes with remarkable superiority compared to the conventional etched Si probes. From the first introduction of such systems as high resolution imaging probes, a lot of effort has been applied to improve the fabrication methodology and to use them as more sophisticated and functional tools. Of particular interest is the fabrication of functionalized "nanoelectrodes" integrated on scanning probes for point-source sensing or triggering of local bioelectrochemical reactions in aqueous physiological environments and with extremely well-defined spatial resolution. As can be envisioned, such issues are becoming very promising due to the huge and increasing interest of the

scientific community in molecular biology and biophysics [44].

Just as an example of the numerous attempts to design functional carbon nanotube probes integrated in a SPM configuration, we can mention our recent work pursuing the fabrication of carbon nanotube based nanoelectrodes [45]. The starting point was a gold coated silicon probe to enhance the conductivity. The "pick up method" developed by Lieber 's group [46] was used to attach the nanotube to the gold coated silicon AFM probe. This consists of scanning the silicon probe in intermittent contact on a silicon substrate coated with single walled carbon nanotubes. During such scanning, the nanotubes can be attached to the gold plated probe by Van der Waals forces. After that, the integrated gold coated silicon/nanotube probe was coated with a very thin conformal Teflon like polymer film formed in an inductively coupled plasma reactor. The polymer mechanically stabilized the attached nanotube and provided a chemically inert and electrically insulating outer layer. Electrical pulse etching of the insulating coating, exclusively at the nanotube tip end, resulted in well-defined highly conductive nanoelectrodes with electrochemical activity limited only to the nanotube tip end. An example of the polymer coated gold Si/Nanotube probe together with the current/potential response can be seen in Fig. 16.

The development of nanoelectrode scanning probes may greatly contribute to the development of a related technique, the scanning electrochemical microscopy. As mentioned be-

fore, this technique allows chemical and electrochemical activity mapping of a substrate based on the faradaic current monitoring of a redox mediator between the substrate and a conductive tip (ultramicroelectrode) [20]. This is an excellent technique for imaging variations in electron transfer rate at metallic electrodes and enzymes in biological materials. However, the disadvantage of the technique resides in the lower spatial resolution reflected by the dimensions of the tip used as ultramicroelectrode as compared to STM and AFM probes. The use of the carbon nanotubes as nanoelectrode could bring about a striking improvement in SECM technology.

Nanomechanical detection using the AFM cantilevers

The force sensing lever is the heart of the AFM system. It responds to external stimuli through deflection in static mode or to changes in resonance frequency in dynamic mode. All these changes can be detected with unprecedented sensitivity. This has opened new research avenues and the increasing development of novel sensors based on SPM technologies [47].

These cantilevers are used as nanomechanical sensor devices for detecting chemical interactions between binding patterns on the cantilever surface and its environment. At the interface between an active cantilever surface and the surrounding medium, the changes in the stress by molecule adsorption, the production of heat, or a mass change, can be detected [47].

The use of cantilever as a platform for experiments with adsorbed molecules has helped to explain processes and reactions in the nanoworld. Cantilever sensor array technique has allowed study of physisorption and chemisorption processes and to determine material specific properties such as enthalpy, phase transitions, etc. Experiments in liquid environments have also given insights into biochemical reactions like hybridization of DNA, molecular recognition in antibody/antigen systems or proteomics.

Summary

Scanning probe microscopies have changed the way we approach the micro/nanoscopic world and to analyze surface phenomena in physics, chemistry, biology, biochemistry and engineering. First designed as techniques to image surfaces with unprecedented spatial resolution, they have extended their capabilities to follow atomic and (bio)molecular processes in different environments and to probe local chemical and physical properties, producing a wealth of related techniques. They have also evolved from a qualitative technique to a more quantitative one. Force spectroscopy has turned out to be a powerful tool to collect quantitative analysis of interaction forces and material properties at selected positions and even at the single molecule level. Tunneling spectroscopy allows the evaluation of the electronic properties of the materials and to distinguish chemically distinct species
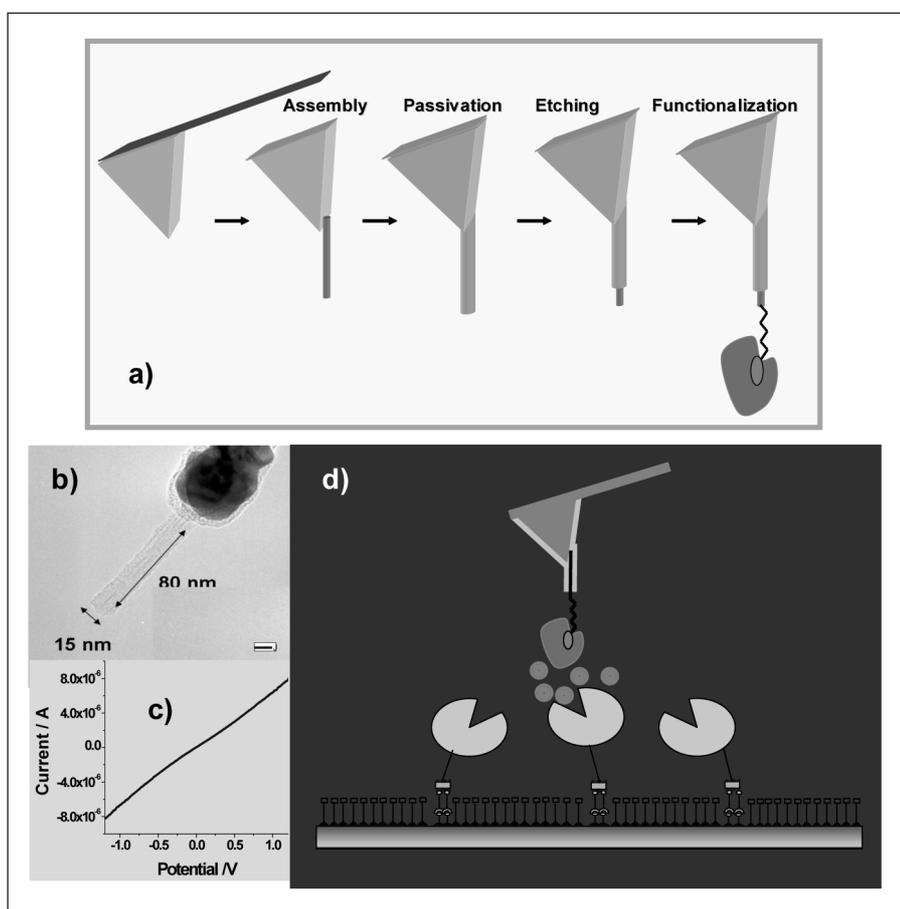

Figure 16. a) Illustrative scheme of the steps used for fabricating functionalized carbon nanotube probes with (electro)chemical activity limited at the apex of the tip. b) TEM micrograph of an 80 nm length single walled carbon nanotube attached to a silicon probe which was previously gold coated. The integrated probe has been covered with a 5 nm layer of Teflon like polymer and the polymer was selectively removed at the nanotube apex by electrical pulses in order to make the probe active as a nanoelectrode. c) Current - potential profile of probe b) in a mercury drop. The nanotube behaves as a conductor with a low bias resistance of 170 kOhms. b) and c) were adapted from Ref. [45]. c) Illustrative scheme of how a biofunctionalized carbon nanotube probe can be used to trigger or sense biochemical reaction on a surface.

based on the measurements of the local density of states and work functions. It can also be used to acquire vibrational spectra of the species. Broad perspectives have been opened up with the capability of SPM to manipulate atom/molecules or induce localized reactions. SPM allows tailoring the chemical composition and structure of a surface on the 1–100 nm length, a striking facility to research topics ranging from electronic conduction, or catalysis, to biological recognition in nanoscale systems. One of the main contributions in this area has arisen from the dip pen nanolithography, a direct write scanning probe– based lithography in which the AFM tip is used as a pen to "write" (deposit) (bio) molecules directly on a surface [48].

Many efforts are in progress trying to make this instrumentation more powerful by coupling it to other techniques, to acquire complementary and more detailed information. AFM has already been combined with optical microscopes including fluorescence imaging/spectroscopy or scanning transmission X-ray techniques.

While the progress in the last two decades has been impressive, some techniques still need to be improved and developed such as the temporal resolution (faster responses especially for studying chemical kinetics), or the proper understanding of the imaging processes (e.g. tunneling / imaging mechanism, phase contrast, cross talk, etc.). In order to increase the resolution and to turn SPMs into a more local sensor and actuator device, the development of SPM probes based on the versatile carbon nanotubes has opened a very promising area. However, it still needs to be explored and exploited. Additionally, the development of nanomechanical sensor devices from SPM cantilevers has proven to be very fruitful. Therefore the continued exploration of these cantilever platforms in order to give further insights into novel (bio)sensor concepts is highly promising.

Finally, the SPM techniques have given birth to a new scientific era marked by the emerging fields of nanotechnology and biotechnology. Without doubt they have provided the key tools for seeing, probing and manipulating the nanoworld, providing new and fundamental insights across a broad spectrum of scientific disciplines.

Acknowledgements

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