Handbook of instrumental techniques from CCiTUB

Atomic Force Microscopy: probing the Nanoworld

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Abstract. Atomic Force Microscope and related techniques have played a key role in the development of the nanotechnology revolution that is taking place in science. This paper reviews the basic principles behind the technique and its different operation modes and applications, pointing out research works performed in the Nanometric Techniques Unit of the CCiTUB in order to exemplify the vast array of capabilities of these instruments.

1. Introduction

Since Richard Feynman sentenced that "there is plenty of room at the bottom" in the 50's, nanoscience and nanotechnology have become two of the cornerstones of modern science. Nanotechnology, thanks to its interdisciplinary nature, is spreading fast in a variety of disciplines comprising, among others, engineering, physics, chemistry and life sciences. Although we usually assume that nano keeps on being a matter of theorists, there are plenty of daily life products that benefit from this new and revolutionary approach. Microelectronics is the most obvious field, as microprocessors and microfabricated parts are becoming smaller and more powerful. Nevertheless, life sciences, medicine and cosmetics are increasingly introducing nanoparticles in common products as solar filters and anti-ageing treatments [1]. These sorts of products have become controversial because they have not been intensively tested in human beings and long-term effects remain unknown [1]. The nanometric diameter of nanoparticles enables them to cross the skin-barrier and entering the blood stream, thus being possibly involved in a variety of cancers. In this uncertain scenario, nanotechnology related industries struggle to show the obvious benefits of their nano-products and to include them in the mainstream market, fact that will change the world we live in forever.

The study of nature in the nanometric range was boosted by the development of the Scanning Probe Microscopes (*SPM*s). The first member of this nowadays prolific family was the Scanning Tunnelling Microscope (*STM*), released in the 80's in the IBM headquarters [2] and famous for producing the first image of surfaces with atomic resolution (Binnig and Rohrer, the fathers of *STM*, won the Nobel prize in 1986). This technique proved to be a cornerstone in the study of catalysis and surface science thanks to its unmatched topographic resolution. Nevertheless, its use was restricted to conductive samples, limitation that soon disappeared with the development of the Atomic Force Microscope (*AFM*) [3], where all kind of samples could be investigated. Despite featuring lower resolution than the *STM*, it was still able to obtain subnanometric resolution in the vertical axis and its obvious versatility spread the number of applications to all kind of scientific fields. Now it was possible to image samples with minimal preparation, both in air and vacuum and also in all kinds of aqueous solutions. Today, it is also possible to perform topographic measurements in a range of temperatures going from 4K (Ultra High Vacuum, *UHV*, operation) up to 250ºC. Nevertheless, *AFM* is more than a way to obtain surface images of matter; it should be considered as a platform to manipulate the nanoworld with unprecedented resolution. In the field of Materials Science, *AFM* can be used as a classic indenter, now with the ability to control the applied vertical force (F_v) down to the tens of picoNewtons level. This is extremely important, for example, to characterize the mechanical properties of thin films and advanced coatings used in the development of Micro- and NanoElectroMechanical systems (*MEM*s and *NEM*s). Of course, this force control represents a breakthrough in biophysics, as now the mechanical properties of cells, proteins and membranes can be experimentally assessed [4]. *AFM* is also able to modify the sample surface by scratching or locally oxidizing it, to probe electric [5] and magnetic properties of samples and to release nanodroplets of molecules in surfaces in order to create selectively functionalized patterns.

Because of this, AFM should be considered as a platform to explore the nanoworld, the core of a system which is being constantly complemented with new operation modes the use of which is step by step demolishing the classic boundaries that still keep Chemistry, Physics, Biology and Engineering apart.

2. Methodology and applications

2.1. AFM basic operation principle

AFM is based on the contact between a microfabricated tip and the sample surface (Fig. 1). As it is a scanning technique, the tip rasters the sample resolving its fine details down to the nanometric level thanks to a tip apex radius ca. 5nm. In a sense, it is the same principle used in a record player, where the needle rides on the grooves of the vinyl record. As the piezo stage proceeds with the

scanning in the XY axis, the cantilever bends up or down (Z axis) and this deflection is detected by means of a laser that finally reflects on a photodetector which tracks the AFM probe response. Usually, the piezo is under the sample, although there are different AFM geometries where the tip is scanned while the sample remains still. Modern AFM piezos cover scanning ranges in the XY plane that goes from hundreds of nanometers to 200 micrometers.

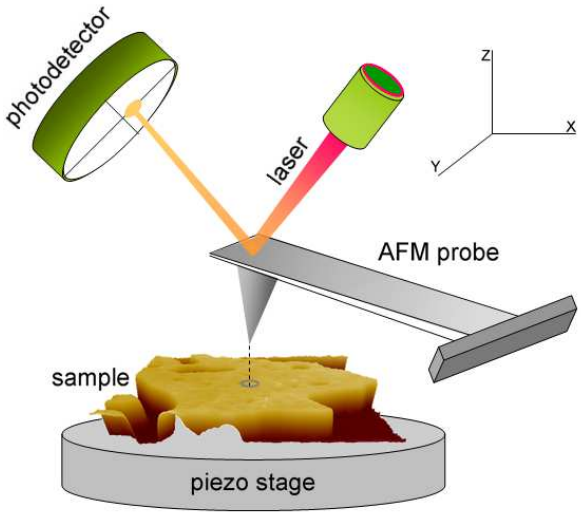

Figure 1. *AFM* schematics. The *AFM* probe tracks the sample surface, which is precisely moved in the XY axis by means of a piezo stage. The deflection of the AFM probe is tracked by a laser that reflects on a photodetector which can detect probe movements in the subnanometric range

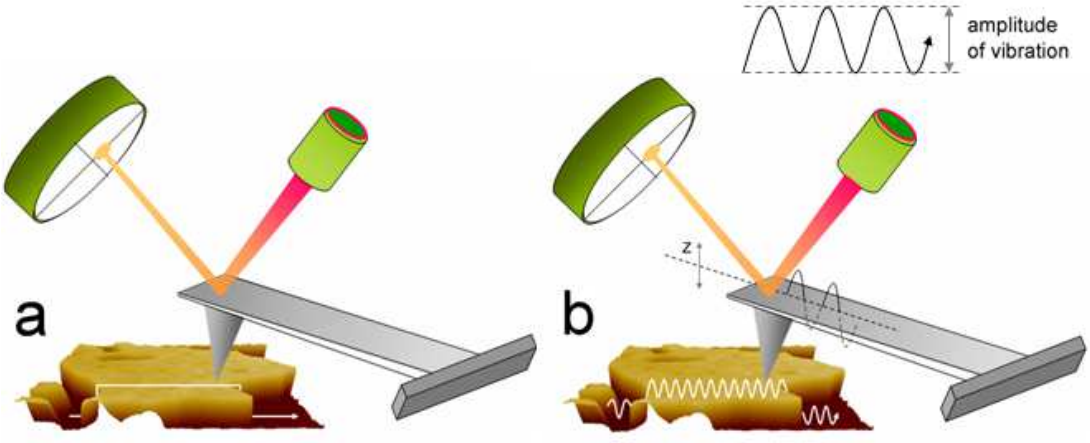

Figure 2. Two basic *AFM* topographic modes. a) In contact mode the *AFM* probe tracks the sample surface in close contact while the feedback loop keeps cantilever deflection constant. b) In intermittent contact mode, the *AFM* probe moves in a sinusoidal way in the Z axis so the contact with the sample is intermittent. In this case, the feedback does not try to keep control on the cantilever deflection but on the amplitude of vibration.

2.2. Topographic modes

2.2.1. Contact mode

In contact mode, the AFM probe is brought into contact with the sample up to a desired cantilever deflection, which results in the application of a certain F_v value. Then, feedback electronics keep the deflection constant as the tip scans the sample in the XY plane by moving the piezo in the Z axis. In fact, this movement corresponds with the sample topography.

This operation mode obtains images with the highest AFM resolution but, due to the direct contact with the sample, it can be aggressive and deform the surface by dragging material away. Besides, the probe radius wears fast due to friction forces and the quality of images can degrade in

a few scans. For all this, contact mode is recommended for atomic resolution topographic images of hard materials (polymers, metals, oxides) [6] or biological samples where structural resolution is mandatory [7] (protein crystals or ion channels on cell membranes).

2.2.2. Intermittent contact mode

Intermittent contact is the most commonly used of all *AFM* topographic modes because it is gentle with the sample surface and also with the *AFM* probe apex, producing consistent data of samples while inducing minimum deformation (Fig. 3e, f). In this mode, the *AFM* probe oscillates at its resonance frequency in the Z axis at a constant amplitude of vibration, which is kept constant by a feedback electronics, while lightly tapping the sample surface. The consequent contact intermittency is enough for the probe to track the sample topography and obtain accurate surface images. Because of this, it is used for imaging all kind of samples, especially biological material [8] both in air and in liquid environment [9]. Some efforts have been performed to improve this mode by combining it with techniques as Infrared spectroscopy (*IR*), *Raman* or *Scanning Near Field Ellipsometer Microscopy* (*SNEM*) [10].

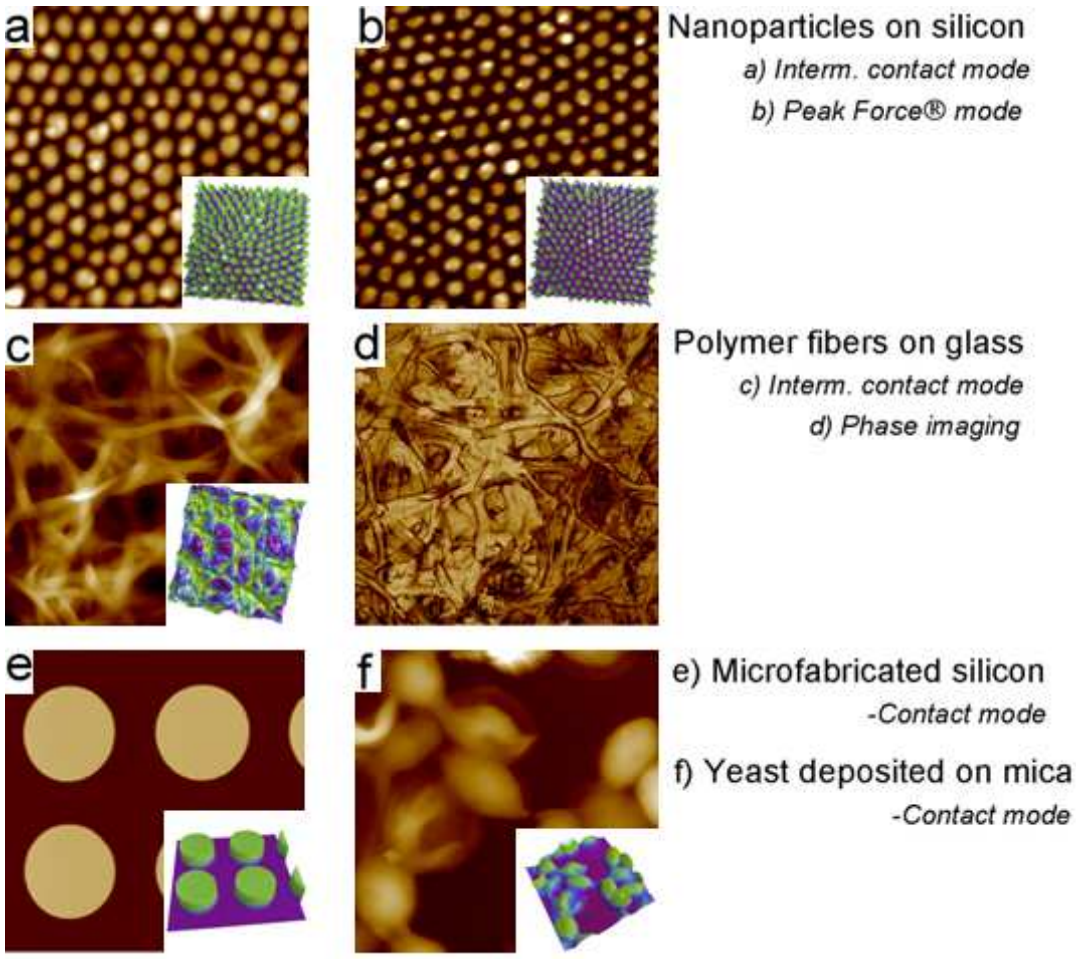

Figure 3. Topographic images obtained by *AFM* in the fields of nanostructures, polymer science, microelectronics and biology. Image sizes: a) and b) 500x500nm^2; c), d) 30x30μm^2; e) 50x50 μm^2; f) 12x12 μm^2. a) and b) correspond to the same sample, demonstrating that Peak Force® is less influenced by *AFM* probe radius, which results in particles with a diameter much closer to reality. c) and d) were obtained simultaneously; d) Phase image shows a compositional contrast which is impossible to detect in topographic image c).

One of the main drawbacks of *AFM* is its lack of compositional sample information. Nevertheless, Phase Imaging (a secondary mode in intermittent contact, Fig. 3d) can provide qualitative data in this direction. This mode is based on the different quantity of energy dissipated by the sample as the tip taps its surface in every vibration cycle. According to this, soft materials will absorb more energy than hard materials, resulting in a phase change of the vibration.

2.2.3. Peak Force® mode

In this mode, the *AFM* probe engages the sample and retracts with a frequency of several kHz. In each cycle, the F_v value is carefully controlled to maximize probe life and reduce sample damage. As this control is fully automatic while the probe scans the sample, Peak Force® is extremely interesting for users, both basic and advanced, that need accurate topographic images. Furthermore, the unprecedented quality of the images due to the low *AFM* probe wear makes it useful when resolution is paramount. An enlightening example can be seen in Fig. 3a) and b), where 10nm diameter nanoparticles deposited on silicon are imaged. a) corresponds to an Intermittent Contact image and b) was obtained in Peak Force® mode. In b) image, particles look much smaller than in a), despite the fact it is the same sample; this is because lateral resolution of images in *AFM* depends on the probe radius. Initially, both topographic modes use the same sort of probes (same final radius) but Intermittent Contact is more aggressive and wears down the radius from the initial 2nm to ca. 5nm to 10nm in a few scan lines. Peak Force® mode is gentler and keeps the initial radius for much longer. As a result, sharper and more accurate lateral resolution is obtained and the measured diameter of nanoparticles is much closer to reality.

2.3. Nanotribology and Nanomechanics

2.3.1 Force Spectroscopy

The *AFM* probe can be used as a nanoindenter to test the mechanical response of samples [11, 12, 13]. Its main advantage is that the measurements can be performed with nanometric spatial resolution and that the F_v values applied on the sample can be as small as tens of picoNewtons ($1pN=10^{-12}N$). Consider a polymeric matrix with embedded nanoparticles; a classic indenter would be able to obtain the Young modulus (*E*) of the whole sample but the *AFM* would be able to perform local nanoindentations both in the matrix and in the nanoparticles independently. These capabilities are especially interesting for biological samples, which have thicknesses usually below 1 micron and where it is extremely difficult to obtain quantitative nanomechanical information by other techniques (Fig. 4). At the CCiTUB, the *E* value of protein aggregates deposited on mica was quantitatively assessed [14] but our expertise has been mainly focused on the nanocharacterization of biological phospholipid membranes and nanometarials. In this field, we have quantitatively measured the F_v value necessary to puncture membranes in different buffer conditions, probing the effect of electrolytes concentration [15] or pH [16] in their stiffness and the influence of the phospholipids structure on their mechanical response [17, 18, 19, 20]. In the field of monolayers, fatty acid and thiols proved to deform under the pressure of the *AFM* probe in a sequential way (step-by-step mechanism), shedding light on the packing structure of these 2D structures [21, 22]. In the field of hard materials, the exploration of obtained *E* values both by traditional methods and AFM nanoindentation are leading to the comprehension of the differences between mechanical properties of surfaces and bulk materials [11, 12, 13]. Other parameters that can be extracted from the so-called force curves, that is, the nanoindentation experiment itself shown in Fig. 4a, are adhesion forces, dissipation energy, the vertical breakthrough force of nanostructures and the force where elastoplastic transitions take place.

Thanks to the Quantitative Nanomechanical Property Mapping Mode (*QNM*) derived from the Peak Force® technology developed by Bruker, all the mechanical parameters can be obtained in real time while capturing a topographic image (Fig. 5). The operation range of this technique goes from tens of MPa (soft biological samples, monolayers, proteins) up to 100GPa (metals, oxides, hard coatings).

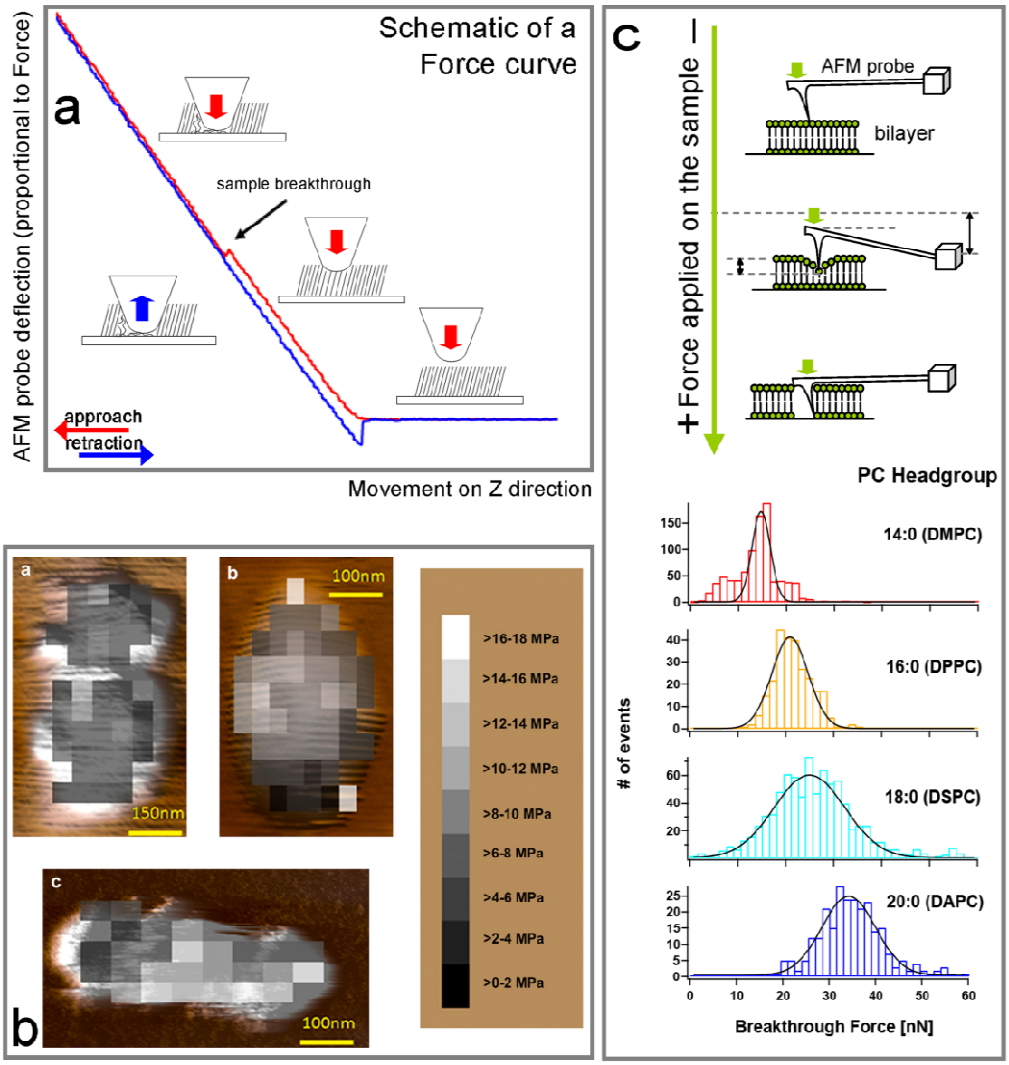

Figure 4. Nanomechanics by *AFM*. a) When a sample is compressed with an *AFM* probe, a force curve is obtained. In the case of mono/multilayered materials, it is possible to quantitatively measure the F_v value necessary to break the sample (shown as a discontinuity in the approach curve in Fig. 4a. b) Different globular proteins imaged by *AFM* where force curves have been performed. A map of Young modulus is over imposed on the topographic images. c) The mechanics of phospholipid membranes can be quantitatively assessed and the breakthrough force measured as a function of the length of the hydrocarbon chains of the phospholipids (in this case, from 14 to 20 carbons).

2.3.2 Friction Force Microscopy (FFM)

FFM is able to quantify the nanotribological properties of a huge variety of samples [23]. These measurements are of great interest when it comes to study materials science-related samples and it is especially useful to characterize the performance of thin films and advanced coatings [24]. In the last years, *FFM* has also been applied to organic films, both in air and in liquid, tapping into phenomena that had never been accessible by other techniques [25, 26, 27]. The operation principle is as follows: the sample is scanned by the *AFM* tip in a direction that is perpendicular to the long cantilever axis (figure 6a). In this way, a cantilever torque momentum is created which can be experimentally measured by following the path of the laser in the lateral quadrants of a four-segment photodetector. The lateral laser deviation is proportional to the friction force (F_f) between the tip and the sample, which in turn is proportional to the F_v value applied by the tip (measured by

the vertical displacement of the laser in the photodetector). F_f value can be measured in a quantitative way in the range that goes from the tens of picoNewtons to hundreds of nanoNewtons. Figure 6b) shows a front view of the cantilever and the tip and how the scanning movement creates a cantilever torsion which in turn is detected as a lateral laser deviation in the photodetector.

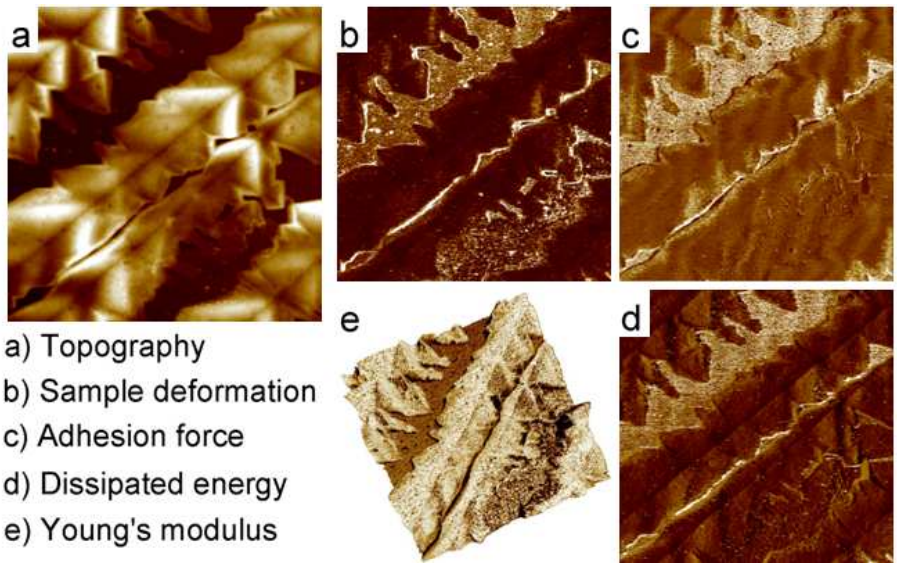

Figure 5. Salt crystals on a mica surface imaged by *QNM*. This mode enables to obtain important quantitative nanomechanical parameters that give information about the physicochemical properties of the sample surface. Image size: 20x20μm^2.

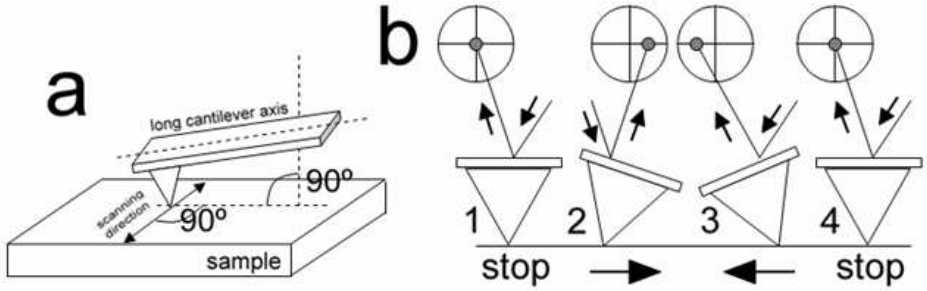

Figure 6. *FFM* operation principle. a) The scanning direction creates a torque on the cantilever which is proportional to the F_f value between the tip apex and the sample. b) The friction response can be measured as a function of the F_v value in order to acquire important nanomechanical parameters as friction coefficient and dissipated energy.

2.4. Electric and magnetic characterization

2.4.1 Conductive AFM (C-AFM)

C-AFM is a secondary imaging mode derived from contact *AFM* that characterizes conductivity variations across medium- to low-conducting and semiconducting materials. The operation principle is simple: a potential is applied between a metal-coated *AFM* tip and the sample; when both surfaces are in contact, an electric current (intensity, *I*) flows across the interface. Thanks to low-noise preamplifiers, *I* values as small as tens of femtoAmpers can be detected. In fact, what we obtain is a map of *I* value besides a topographic image [28]. This is of important relevance for the microlectronics and photovoltaics industry but also for the development of new nanometarials as carbon nanotubes [29] and graphene [30], where conductivity of extremely small parts of integrated circuits has to be measured. The variations in *I* map indicate the presence of defects in the

semiconductor materials, the control of which are mandatory in fabrication processes. Besides, the ability to control the position of the AFM tip in the nanometric range enables the user to stop the tip on a certain point of the sample surface, apply a desired F_v value and ramp the potential in order to obtain an extremely local *IV* curve.

2.4.2 Magnetic Force Microscopy (MFM)

Magnetic Force Microscopy (*MFM*) is a secondary imaging mode derived from Intermittent Contact topographic mode that maps magnetic force gradients above the sample surface. Thanks to a tip coated with a magnetic alloy, the topographic and magnetic images of the sample can be simultaneously obtained. The extremely high resolution of this technique enables the study of molecular magnets, as well as magnetic thin films [31] (Fig. 7).

MFM probes tip is coated with a ferromagnetic thin film. While scanning, it is the magnetic field's dependence on tip-sample separation that induces changes in the cantilever's resonance frequency or phase. *MFM* can be used to image both naturally occurring and deliberately written domain structures in magnetic materials (Fig. 7a and b).

2.4.3 Surface Potential (SP), also known as Elestrostatic Force Microscopy (EFM)

SP is a secondary imaging mode derived from Intermittent Contact topographic mode that maps the electrostatic potential on the sample surface. As the *AFM* probe has to be sensitive to electric charges, it is necessary to coat it with a metal or conductive layer as doped-diamond (highly wear-resistant). It is interesting to note that the sample can be an insulating because the only charges that are detected are on the surface; no electrons flow across the interface, as no voltage difference is applied between the *AFM* probe and the sample. This technique can be applied to detect the very subtle charge differences that arise when an organic monolayer is being formed on a substrate or to identify different domains in mixed phospholipid bilayers. It is worth to note that a slightly refined mode called Surface Potential Force Microscopy (*SPFM*) [32] is able to scan the sample in real non-contact while obtaining images of liquid droplets, being of special interest for the study of 2D lubricants [33] and corrosion science [34].

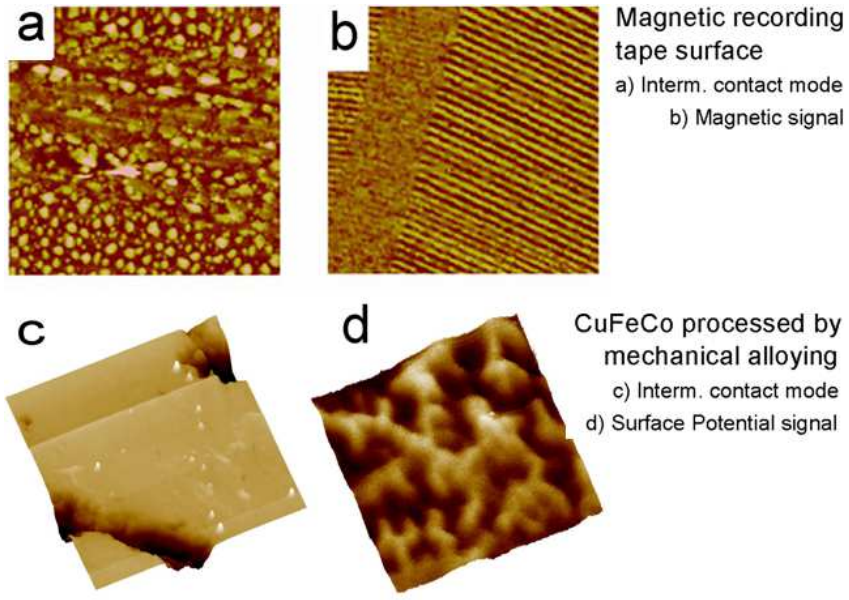

Figure 7. The ferrite grains present on the surface of a magnetic recording tape can be topographically resolved (a) and, at the same time, the magnetized domains are revealed by *MFM* (b). Notice that there is no cross-linking between the topography and the magnetic domains. c) and d) correspond to the topography and surface charge distribution signal (*SP*) on a metallic sample. Although topographic image reveals a smooth surface, *SP* signal is able to discern different charge domains which can be related to compositional gradients.

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Glossary

SPM. Scanning Probe Microscope
STM. Scanning Tunnelling Microscope
AFM. Atomic Force Microscope
UHV. Ultra High Vacuum
F_v. Vertical Force
*MEM*s. MicroElectroMechanical Systems
*NEM*s. NanoElectroMechanical Systems
SNEM. Scanning Near Field Ellipsometer Microscopy
E. Young's modulus
QNM. Quantitative Nanomechanical Property Mapping
F_f. Friction Force
FFM. Friction Force Microscopy
C-AFM. Conductive AFM
I. Current Intensity
V. Potential
SP. Surface Potential
SPFM. Surface Potential Force Microscopy
EFM. Electrostatic Force Microscopy

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