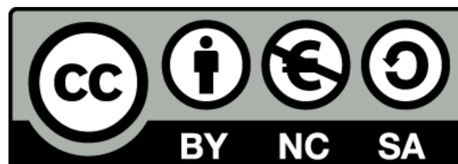
Electric polarization properties of single bacteria measured with electrostatic force microscopy

Theoretical and practical studies of Dielectric constant of single bacteria and smaller elements

Daniel Esteban i Ferrer



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UNIVERSITAT DE BARCELONA

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Barcelona, September 2014

DOCTORAL THESIS

3 Basics of Atomic Force Microscopy and Electrostatic Force Microscopy

The Atomic Force Microscope lies in the field of the called Scanning Probe Microscopes (SPM). Other instruments within this field are the Scanning Tunnelling Microscope (STM), or the Near-Field Scanning Optical Microscope (NSOM/SNOM), which can image a surface resulting on a three-dimensional topography, with a resolution down to the atomic level. Some of these microscopes have attached capabilities to measure other physical or chemical magnitudes of the sample. They are composed mainly of three-parts, the scanner, the probe and the processor. The probe is put into close proximity (nanometer level) until a certain interaction (depending on the sensors) is obtained on the sharp tip at the end of the probe. This interaction is processed by the processing unit which makes the scanner to react, usually by acting on a piezoelectric ceramic (piezo) to maintain the interaction constant (in the Z direction usually). This is the case of the closed loop mode, although one can use the open loop mode where the piezo is actuated regardless a certain set point and the resulting interaction is recorded. At the same time, and as they are scanning techniques the X and Y direction are also moved by acting on the piezos that move these directions (one will be the fast, and the other the slow since the scanning is done line by line) at a certain speed and resolution. A simplified scheme of a SPM can be seen in Figure 3.1.

The first SPM to appear was the STM back in the early 80’s [18] for which his inventors were awarded with the Nobel Prize in 1986. It is based in the tunnelling current from a very sharp probe to the sample, so both the sample and the probe must be conductive (or very thin insulating).

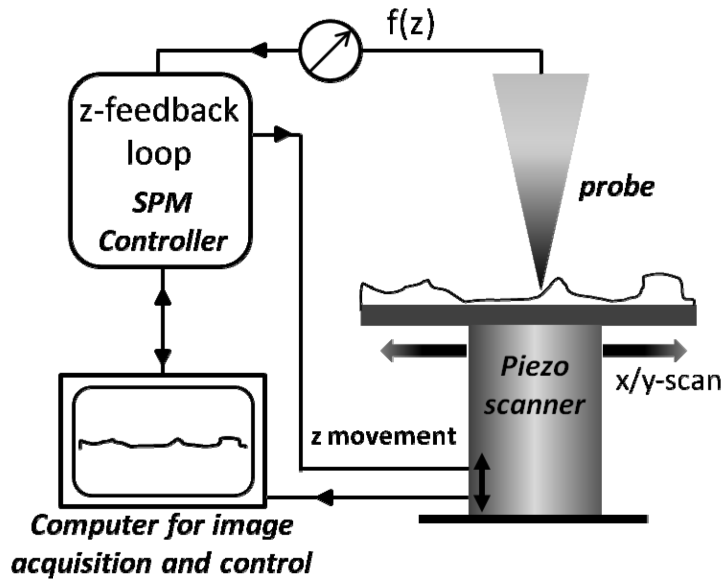

Figure 3.1 *Simplified set up for a scanning probe microscope. The piezo the sample laterally and adjusts the tip sample distance, z. The probe senses the sample and gives a signal that is dependent on the probe-sample distance. The control and feedback circuit maintains the probe-sample distance so that a surface image can be acquired. (Image courtesy of G. Gramse, reproduced with permision).*

This current goes exponentially with the proximity to the sample, so a current feedback will maintain a certain distance. The displacement of the piezo scanner will be recorded giving the topography of the sample.

As an evolution of the STM the AFM appeared in 1986 [19] firstly based on a (very small 100-400 μm long) cantilever whose deflection was measured using an STM. The STM was later substituted for simplification with a laser and a multiquadrant photodiode. The cantilever has a sharp tip in the free-end whose apex is in the nanometer range (1-200 nm). This tip is what causes the cantilever deflection when it interacts with the sample. The AFM opened up a whole new world of possibilities as the simple set-up and feedback (the laser deflection) is able to perform measurements of insulating samples,

in ambient conditions and in liquids and depending on the used cantilever (functionalization) a lot of different measurements can be performed. Besides forces, in the pN range (van der Waals, capillary, chemical, electrostatic, etc.), other quantities can be measured such as (thermal, joule, phototermal, etc.). That is why the AFM is so versatile and can be used from material science to molecular biology including chemistry, pharmacology and others.

SPMs can be also built with the help of other sensors, such as a tuning fork, in which case the feedback is either on the oscillation amplitude or the resonance frequency of a self-oscillating small quartz tuning fork. These quantities change also with the interaction between a tip mounted on the tuning fork and the sample.

The NSOM/SNOM is based in one of the previous feedback SPMs (AFM, SPM-TF), where the tip that is put close to the sample is modified so it can interact with the light that can be either illuminated from the tip, from outside of the tip or from the sample, giving different modes of operation at sub-diffraction limit.

There are many other SPM techniques with different feedbacks which measure infinitude of magnitudes but in the present thesis we will be using the AFM for topographic measurements and the Electrostatic Force Microscope (EFM),), which is an adaptation of the AFM that also allows electric force measurements to be carried out. We describe them in more detail in the forthcoming sections.

3.1 Atomic Force Microscopy: topographic modes

The AFM has been the base for many other SPMs by modifying the feedback and/or the magnitude probed. But the first and still the most common use for the AFM is the acquisition of topography of the sample surface. The AFM is based on the short range forces that appear when a probe is approached to a sample surface. These forces are initially attractive and of the van der Walls type ($z < 10$ nm from the sample), which produces the negative deflection of the cantilever

towards the sample (see Figure 3.2 non-contact regime). When the probe is further approached, then the interaction starts to be repulsive and when it is pushed farther than the zero-deflection point it enters the contact regime (Figure 3.2) which starts to apply some force to the sample (this point is crucial because when soft samples are used they can be modified).

Depending on the force range in which the probe is located different imaging modes can be defined, as detailed in what follows.

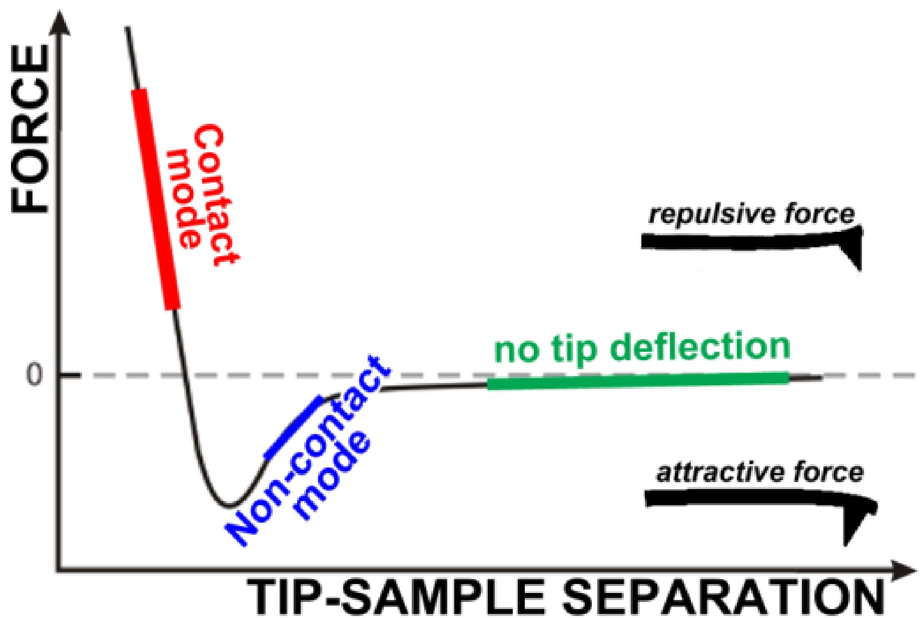

Figure 3.2 *Interaction regimes for Atomic Force Microscopy. At large separations when the sensed forces are just attractive images are acquired in non-contact. At close distance and repulsive forces – contact mode. Covering both ranges - dynamic contact mode.*

3.1.1 Contact Mode

In this mode the cantilever is in the repulsive region. When the force applied is zero the deflection is also zero, but it can be very unstable when the feedback values are not well set or the scan velocity is too fast. That is why it is usually operated with some deflection and soft cantilevers ($k<2$ N/m) to avoid damaging the sample. The processing unit (using the feedback algorithms) will keep a constant force applied using the piezo to raise or lower the probe depending on the topography. This piezo movement together with the X and Y movements are recorded point by point (depending on the established resolution there will be more or less points). This recording gives topography of what are the changes in height of the observed sample.

3.1.2 Dynamic Mode

In the case of the dynamic mode the cantilever is mechanically oscillated by a small piezo usually attached at probe holder. To maximize the amplitude applying the minimum energy the cantilever is usually operated close to the resonant frequency. There are other ways to excite the cantilever such as using magnetic fields, or by heating using the laser. These modes are interesting for liquids since they do not (or minimally) excite the liquid mechanically.

Once the cantilever is oscillating it is brought to contact using the Z piezo. To know that the tip is close to the sample the amplitude is monitored and the set point is typically set at 60-70% of its free oscillation amplitude. As it is intermittently going from contact to non-contact regime, the sample is just slightly touched and hence tips and sample modifications are less evident.

In this case the amplitude is used as the feedback to move the piezo in the Z direction to compensate for the amplitude change in each point. Again (as in contact mode) the movement of the piezo is recorded giving the differences in height of the observed sample (see *Figure 3.3*).

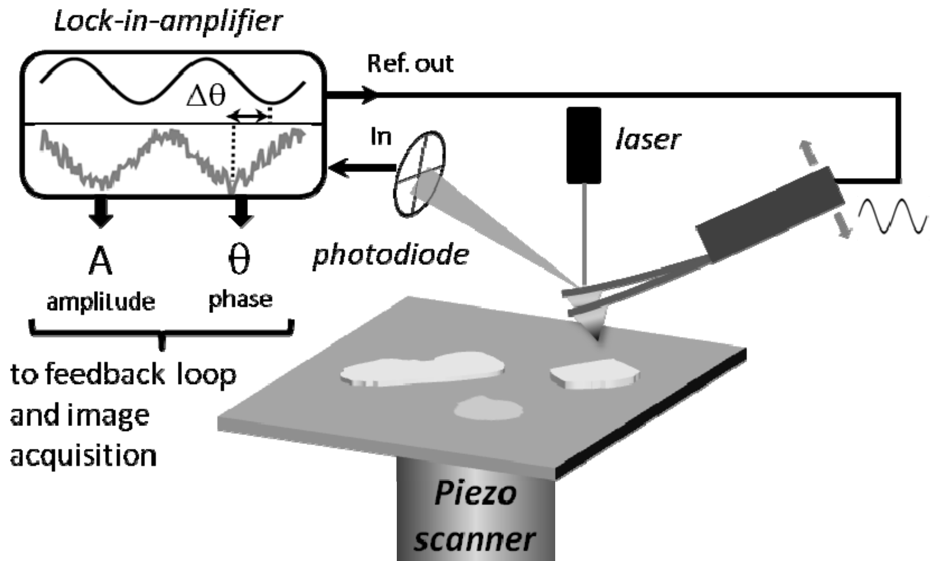

Figure 3.3 *Set up for amplitude modulation AFM. The cantilever is excited mechanically to oscillate at its resonance frequency. The oscillation is monitored by the deflection of the laser spot on the photodiode. A lock-in amplifier detects from this oscillation the amplitude and phase of the cantilever oscillation that change when getting into contact with the sample. The amplitude signal is used to maintain the probe sample distance and acquire a topography image. (Image courtesy of G.Gramse, reproduced with permission).*

In the dynamic mode, also the phase change of the oscillation is acquired thanks to the lock-in amplifier (by comparing the excitation with the actual vibration). This can be useful information to get some material properties such as, composition, elasticity, viscosity, visco-elasticity, among others.

3.1.3 Non-Contact Mode

This is a dynamic mode by in which the whole oscillation takes place in the non-contact region. Usually, the effect of the attractive forces is detected through a shift in the resonant frequency of the cantilever. In this mode, when the tip is close enough to the sample it gets attracted by the van der Waals forces of the surface. They depend on the distance to the sample, so by obtaining the frequency shift or amplitude shift (using a phase locked loop) the feedback control can modify the height of scanning (helped by the piezo) which is later recorded as the sample surface topography.

In this mode neither the sample nor the tip are modified but since the forces sensed are weak the feedback mechanism is in precarious condition and is hard to maintain.

3.2 Atomic Force Microscopy: electrical modes

One of the uses of the AFM is in the measurement of the local electrical properties of samples, such as, impedance, current (classical or tunnel), dielectrical polarization, surface potencial, etc. Usually the substrate and the tip/probe has to be conductive (or semi-conductive) although the sample can be isolating and much thicker than in the case of STM.

First three examples of current detection techniques are presented. In them current flowing through the tip is measured and hence electrical properties related can be accessed. These techniques are Conductive-AFM, Scanning Capacitance Microscopy (SCM) and Nanoscale Impedance Microscopy (NIM).

Conductive AFM (C-AFM)

It is similar to a miniaturized multimeter to measure the conductivity of a circuit, when applying a DC bias voltage between the tip and the substrate. The I_{dc} current is then measured using an amplifier (since the

current is very small) in each point thus giving an overlapped topography-conductance map. The necessity to have a good contact with the surface implies high forces (deflections) thus being best suited for hard materials with hard tips (i.e doped diamond) and for instance in the semiconductor industry.

Scanning Capacitance Microscopy (SCM)

SCM is another technique broadly used in semiconductor industry to obtain the differential capacitance (dC/dV) simultaneously to the topography with nanometrical resolution. To do so GHz resonant circuit is formed with the tip sample being one of its capacitive elements. Changes in the tip sample capacitance induce changes in the circuit resonance frequency which can then be measured. This technique is typically used with semiconductor samples, in which a low frequency potential is superimposed to modulate the space charge capacitance and obtain information on the sample doping density.

Nanoscale Impedance Microscopy (NIM)

Most recently the NIM has been developed [20]. It is similar to an impedance analyzer but at the nanoscale. It measures the impedance $Z(\omega)$ of the sample with nanoscale lateral resolution (10 nm) and it can be done mapping the surface at the same time as the topography. The measurement can be done at a fixed frequency or in spectroscopic mode (doing an impedance spectroscopy at each point). A similar approach using a low noise current-to-voltage amplifier together with a lock-in detector has been used by our group to measure oxide thin films [21] [22] [23] and even a 5 nm height biomembrane [24] in non-contact mode and also with a lateral resolution of about 10 nm. In this case the difference in capacitance of the nanometrical domains is much smaller than the stray capacitance of cables, and so there must be a very sensitive low noise amplifier in order to resolve such quantities. The differences in the case of the biomembrane are in the order of the 1aF [24].

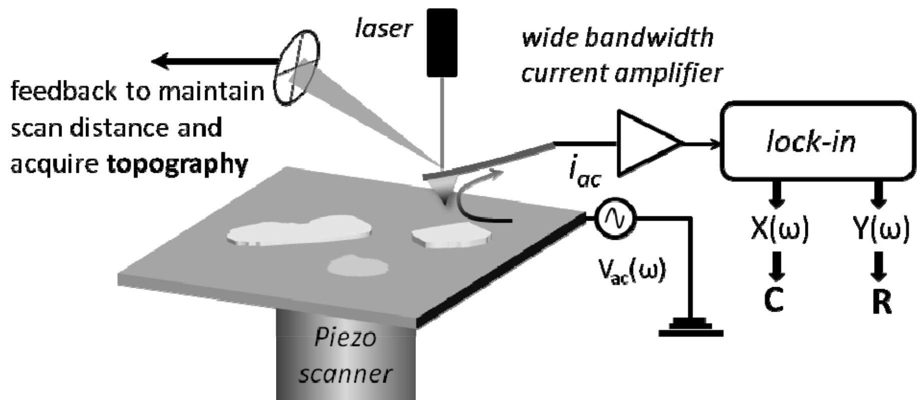

Figure 3.4 *Experimental set up for Nanoscale Impedance Microscopy (NIM) with the implementation consisting of a wide bandwidth current amplifier and a lock-in to demodulate resistive (X) and capacitive (Y) current. (Image courtesy of G.Gramse, reproduced with permission).*

A second set of electrical techniques are based on the measurement of the electrical force sensed by the probe which depends on electrical properties of the sample. Three more examples will be exposed in the next paragraphs, although Electrostatic Force Microscopy (EFM) will have its own section (3.3) since it is the chosen technique for this thesis and will be further expanded.

Kelvin Prove Force Microscopy (KPFM)

KPFM is a kind of microscope to measure the surface potential (surface charges) of a sample which makes it very interesting in biological, organic and inorganic interfaces (i.e semiconductor industry), etc.

In this case an electrical excitation is applied to the cantilever which oscillates at a certain frequency. The first harmonic of the electric force can be shown to be proportional to the difference between the contact potential and a DC applied voltage. Therefore, this mode can be minimized and nulled by the use of a DC-bias potential to the sample. This DC-bias corresponds to the surface potential when the first harmonic's amplitude is zero.

Scanning Polarization Force Microscopy

This is a very useful technique to study thin soft layers like water on (mainly conductive) substrates [25] [26]. It is similar to the previous type of microscopy but in this case the second harmonic amplitude (related to the polarization force) is feed to the feedback so that it is kept constant. In this case the image obtained includes the sample topography correlated to the dielectric sample response, which makes impossible to uncouple them unless one of the magnitudes (height, or polarization) is known, but still can be used as a very useful imaging technique for very soft samples.

3.3 Electrostatic Force Microscopy

The experimental part of this thesis is based in the Electrostatic Force Microscope (EFM) and its capability to measure the electrostatic interaction between a sharp conducting tip and a sample. This technique has benefits to study biological samples (i.e biomembranes, viruses, bacteria, etc) from its potential to perform electrostatic force measurements in a non-contact label-free manner. There are mainly three main ways to work out with the EFM, namely, with constant applied volatge (DC-EFM) [27], in amplitude modulation (AM-EFM) [28] [29] [30] and in frequency modulation (FM-EFM) [31]. From now on over the entire thesis (unless otherwise stated) the amplitude modulation will be chosen for its good resolution and relatively simple implementation and interpretation. In this mode an alternating potential

$$V = V_0 \sin(\omega t) \qquad (3.1)$$

at frequency ω is applied between tip and substrate. The applied voltage creates a static deflection, F_{dc}, another force oscillating at the first harmonic of the frequency F_{ω} and a force oscillating at the second harmonic $F_{2\omega}$:

$$F_{dc}(z) = -\frac{1}{2}\frac{\partial C_T(z)}{\partial z}\left[\frac{1}{2}V_{ac}^2 + \left(V_{dc} + V_{sp}\right)^2\right] \quad (3.2)$$

$$F_{\omega}(z) = -\frac{\partial C_T(z)}{\partial z}\left(V_{dc} + V_{sp}\right)V_{ac}\sin(\omega t) \quad (3.3)$$

$$F_{2\omega}(z) = \frac{1}{4}\frac{\partial C_T(z)}{\partial z}V_{ac}^2\cos(2\omega t) \quad (3.4)$$

$F_{2\omega}$ depends only on the tip-sample capacity, and hence it can be used to measure the dielectric constant of the sample, although it also depends on the geometry of the probe and the sample. So a methodology of calibration has to be performed (see chapter 4), and the geometry of the sample needs to be accounted for. Besides this if the sample has some internal structure (e.g. it has different dielectric constants) a model has to be proposed to account for it, as we have done for the case of bacteria [28] (chapter 4.5) and viruses [29] (chapter 7.1), where shell-core models have been developed, that in the case of the viruses can also be very dependent on the sample eccentricity [32].

The basic mechanism to detect the forces at F_{ω} and $F_{2\omega}$ can be seen in *Figure 3.5*. A lock-in amplifier excites an electrical oscillation to the cantilever by applying the ac-voltage at frequency ω (much lower than the mechanical resonant frequency). This oscillation is acquired by the photodiode and the amplitude at the first and second harmonic ($A(\omega)$, $A(2\omega)$) of the excitation frequency comes back to the lock in amplifier which filters 1st and 2nd harmonic separately. Since the Lock in amplifier is very selective in frequency a huge reduction in noise is achieved (this is one of the reasons why AM-EFM is used instead of DC-EFM). From the oscillation amplitude and calibrated cantilever spring constant, the electrostatic force, and hence, the capacitance gradient, can be obtained. The permittivity of the sample can be obtained from constant height measurements at different heights or by approach (force distance) curves done at different points. Both methods should give (and in fact they do) the same results as it will be seen in chapter 4.

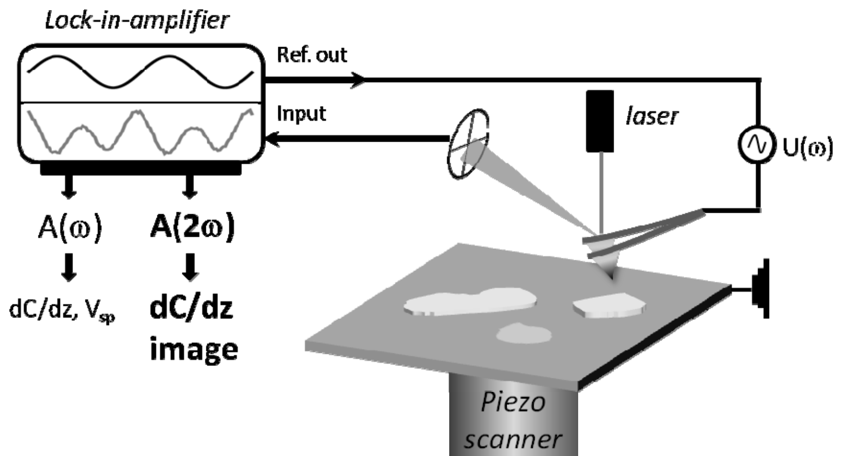

Figure 3.5 *Experimental set up for Amplitude Modulation Electrostatic Force Microscopy (AM-EFM). (Image courtesy of G.Gramse, reproduced with permission).*