
# 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

UNIVERSITAT DE BARCELONA

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**Barcelona, September 2014**

DOCTORAL THESIS

# 4 Validation of Quantitative Electrostatic Force Microscopy on silicon nitride calibration samples

## 4.1 Abstract

It is demonstrated that the procedure derived by our research team to measure the local dielectric constant of materials can be applied to dielectric materials of intermediate thicknesses in the range (100nm -1000 nm). The procedure is tested on a silicon nitride ($Si_3N_4$) dielectric layer giving a relative perittivity of $\varepsilon_r$ = 7.65, in excellent agreement with the nominal permittivity of ~6-8. The procedure is considered validated and hence ready to be used with biological samples of similar thicknesses (e.g. bacteria cells).

## 4.2 Introduction

A good way to prove that a methodology in science is correct is to use it to measure a known sample (name it a calibration sample) so that if the answer is the expected one we can assume that the procedure is correct. Once it is proved it can then be applied to unknown systems and trust on its response, thus obtaining new data which can be taken with a high degree of confidence. This is the case of our methodology, experimental and theoretical to measure the dielectric constants of different materials (see chapters 5 and 6). The dielectric constant of a material can be a very interesting magnitude since it is an intrinsic property of matter and could be used as a fingerprint to distinguish, catalogate, differentiate, finding new properties, etc. for inorganic or

organic materials (also could be very useful for biological samples as we will show in this work of thesis). In our case we are going to use a calibration sample consisting of a dielectric film (thickness around 250 nm in the range of bacteria height) of Silicon Nitride ($Si_3N_4$) over a conductive gold (Au) substrate. The dielectric layer is micromachined in trenches 10 μm wide and several μm long (see *Figure 4.1*). The length and width of the trenches are high enough to to avoid lateral finite size effects.

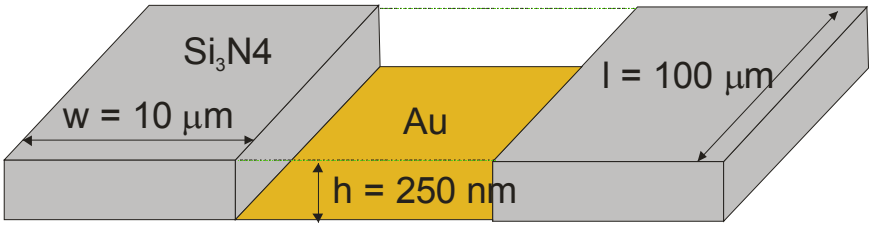


*Figure 4.1* *Schematic of the calibration sample. Long trenches (100 μm) of $Si_3N_4$ are deposited over a conductive gold substrate. The width of the trenches are 10 μm and the height 250 nm. The pitch is also 10 μm (not to scale).*

In the appendix a figure of a similar procedure done on thin silicon oxide ($Si0_2$) with the same procedure is shown.

## 4.3 Measurement protocol and theoretical model

The measurement protocol followed here is the one described in chapter 5. That is, I mounted a conductive coated doped diamond tip (CDT-CONTR) in the tip holder of the AFM. The calibration sample was glued using silver paint to a conductive magnetic support. A cable was glued to the support (also by silver paint) and connected to a conductive screw to sample stage of the AFM. This stage has a connector to set it as zero potential. The ground connection was made through a large resistivity (several gigaohm's resistor) to avoid large current circulation

that could damage either the tip or the sample when in contact.

The tip bias was connected to the lock in output (through the breakout box) to apply an AC current when necessary and the deflection output of the laser was fed back to the lock in to obtain the oscillation amplitude (AM modulation) at double the primary electric frequency. The obtained data was then fed back to one of the auxiliary inputs of the breakout box so that the data would properly be recorded.

Once the head was mounted and laser aligned I mechanically approached the substrate using the optics to see when the tip was close enough to the sample. Then the mechanical excitation (at frequency near resonance – dynamic mode) was applied and the tip was automatically approached (by the piezo) until the desired amplitude set point was achieved. Then the sample was scanned and by moving the piezos the tip was situated at the desired place (see Figure 4.2). The height of the step was 263 nm and the scanning size was larger than 10μm x 10μm.

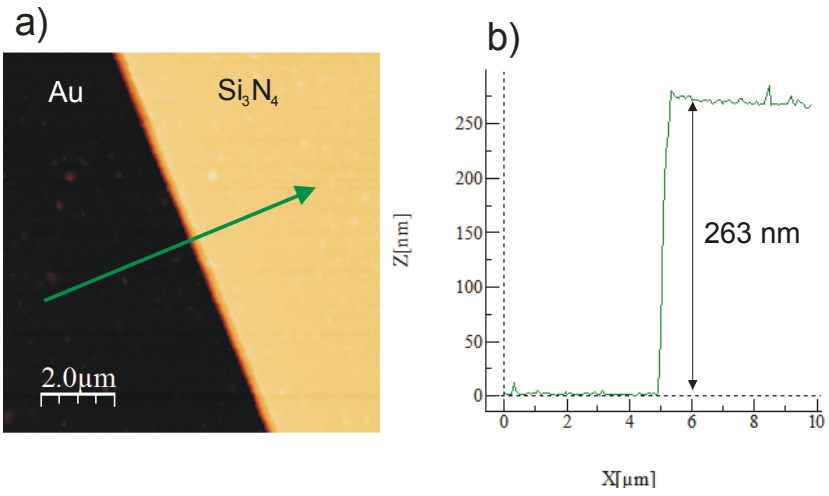


*Figure 4.2* *(a) Topographical image (in dynamic mode) of the sample and (b) crossectional profile taken along the arrow in (a). The height is 263 nm and the scan size about 10 μm x 10 μm.*

After this I disconnected the mechanical excitation and connected the electrical excitation (4-5 volts at 2 kHz frequency) and several force

distance curves were recorded over a line in the gold substrate and in the dielectric ($Si_2N_4$). The regions farther apart were used for tip calibration and for dielectric constant extraction (to avoid lateral finite size effects). See Figure 4.3 for a visual representation.

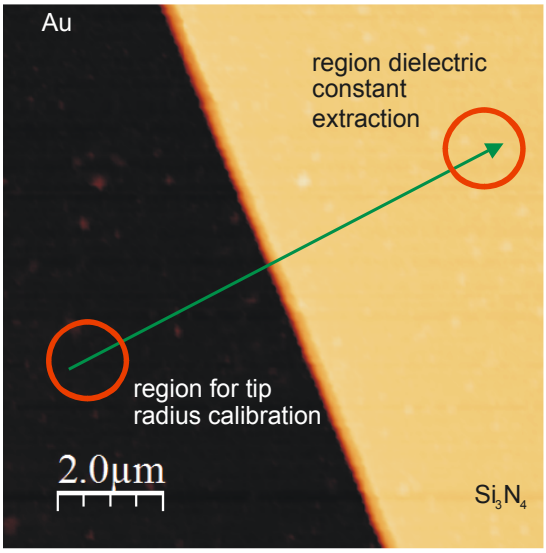


*Figure 4.3* *The red circle areas are the places were the tip calibration and dielectric constant extraction curves are taken for analysis. N.B: both regions are far apart from the interface region to avoid finite size effects.*

The force distance curves were converted to the desired units (zF/nm) by taking into account the corresponding gains. The metal region was used for tip radius calibration (the value at 200 nm was used as reference) see Figure 4.4 for an example. The tip calibration procedure is explained in the chapter 5.

With the calibrated tip and the obtained topography (h and fixed w = 10 μm) simulations for different permittivities are realized. With this data using fitting algorithms we programmed using Matlab, the dielectric constant was obtained for about 10 lines (N=10) over the dielectric substrate. The reference was taken in metal with a height of h

+ z and later subtracted to the curve in the dielectric ($\Delta C'(z)$).

The approach curves were later on averaged and the mean and standard deviation was taken as the measured value. In Figure 4.5 we see the fitted curve red line giving a permittivity of 8.2 +/- 0.6.

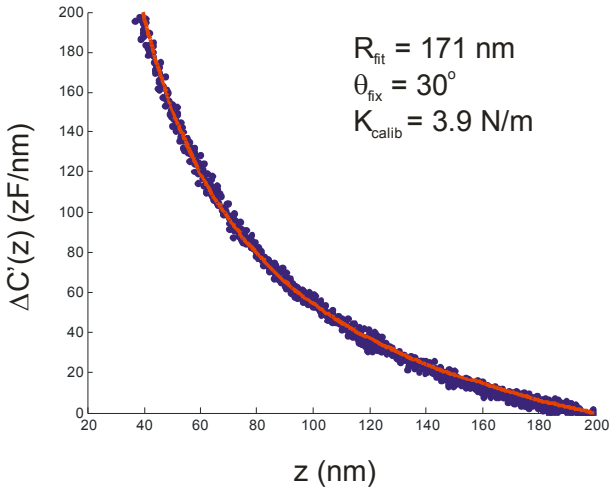


*Figure 4.4* *Example of a tip radius calibration. The obtained tip radius (R) is 171 nm, and the cone angle aperture ($\theta$) was fixed to 30°. The spring constant (K) was calibrated using thermal noise method giving 3.9 N/m. The symbols are the force distance curves obtained in the metal region (N=10). The red solid line is the fitted curve.*

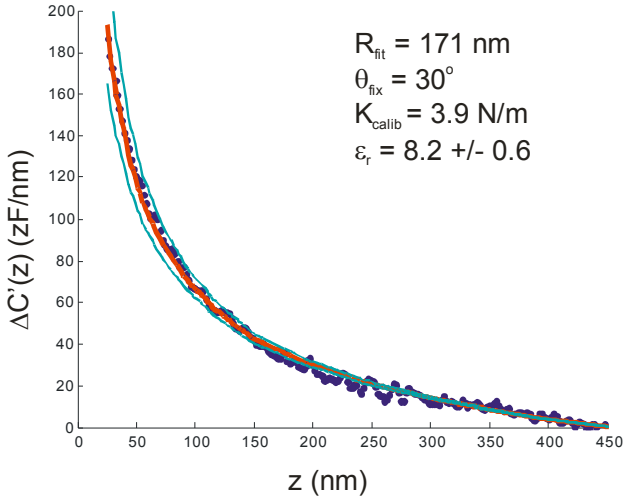

*Figure 4.5* *Example of a dielectric constant extraction. With the calibrated values (R=171 nm, q=30o, K=3.9 N/m). The symbols are the force distance curves obtained in dielectric region. The red solid line is the fitted curve giving 8.2 +/- 0.6 in this case.*

The same procedure was repeated three times, in different regions, and with different tips giving R = 67 nm, K = 3.7 N/m, $\varepsilon_r$ = 7 +/- 1 and R = 164 nm K = 3.8 N/m, $\varepsilon_r$ = 7.6 +/- 0.2. The final value averaging all of them is 7.6 and the nominal value is 6-8, with a perfect agreement between the two values.

## *4.4 Constant height vs. force-distance curves*

In our research group we have used also the constant height mode which implies a different approach. The topography is done the same way in dynamic mode and once it is obtained a certain script is run which obtains constant height electric images with respect to the metallic substrate. In our case the nanosurf Easyscan 2 control software has an implemented constant height function to do so. The way it works is as follows:

i) The tip is placed in the upper left corner with its set point (in contact mode).
ii) The feedback loop opens (free mode).
iii) The piezo is retracted a certain amount chosen by the user.
iv) The piezo scans one line, forward (to the end of the scan size) and one line backward.
v) The piezo is approached the same amount chosen by the user.
vi) The feedback loop closes again and the scanner waits until the set point is reached.
vii) The tip goes to the next line in the slow direction.

viii) The process starts again from iii) until the whole image in constant height is completed.

In Figure 4.6 the topography of a region with silicon nitride and gold (a). The crossection marked with a green arrow can be seen in (b) with a height of 263 nm. By doing the constant height mode we obtained the contrast of the capacitance gradient shown in Figure 4.6c. The values of the potential can be seen in (d), where the potential of each pixel is added to a histogram that ends up with two peaks (as expected). By fitting the peaks with a Gaussian function we obtain the difference in potential. The gain of the whole system is 130 (zF/nm/V) which gives us the $\Delta C'(z)$ (Figure 4.6d).

Following the same procedure at different heights and comparing it to the obtained force distance curves we see a perfect agreement of both approaches (Figure 4.7).

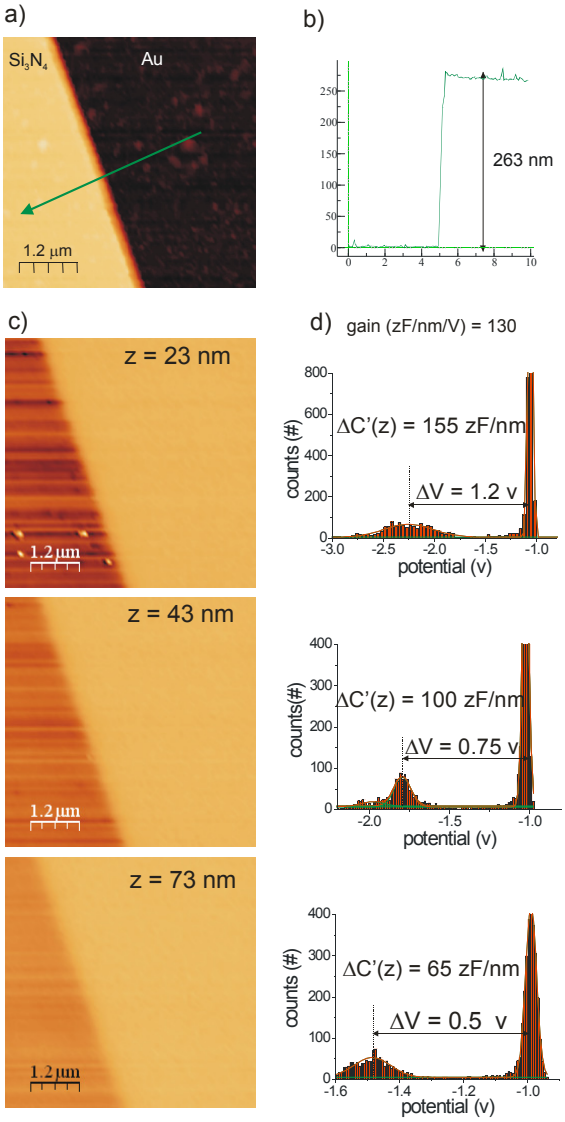


*Figure 4.6* *(a) Topography and (b) crossection of the of a silicon nitride step over gold. The height is 263 nm. In (c) three different constant height capacitance gradients taken at z = 23, 43, 73 nm. In (d) the two peaks of the capacitance gradient and the resulting difference.*

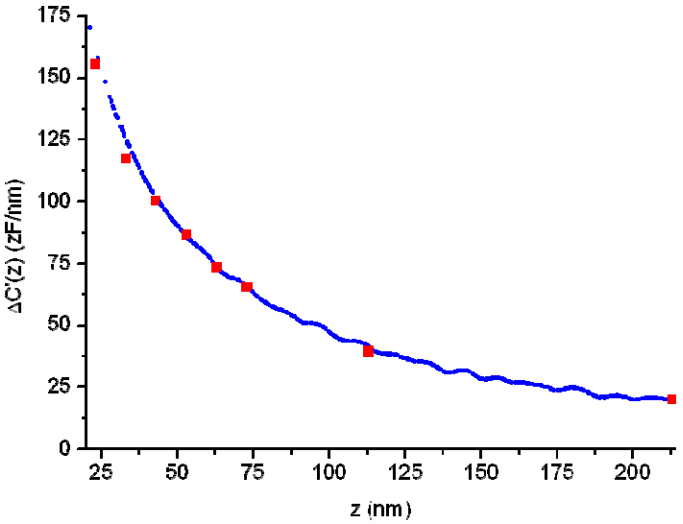


*Figure 4.7* *Capacitance gradient difference ΔC'(z) using force distance (blue symbols) and constant height (red squares) approaches. The agreement is perfect.*

## 4.5 Appendix

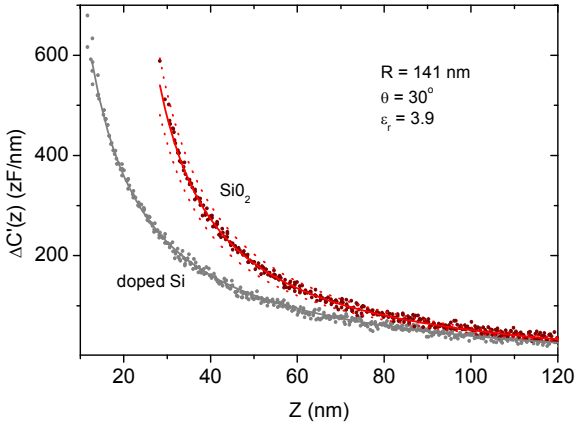


*Figure 4.8* *Doped silicon approach curve (grey symbols) with a fitting R = 141 nm (grey solid line). Approach curve in $SiO_2$ (red symbols) getting a permittivity of er= 3.9 (the fitting is the red solid line).*

## *4.1 Conclusion*

By using the force distance procedure to a calibration sample of silicon nitride over gold we obtained a permittivity of the dielectric of $\varepsilon_r$ = 7.6. The nominal value is 6-8 thus validating the procedure in both, the theoretical framework and the experimental methodology. Also a permittivity of $\varepsilon_r$ = 3.9 ($\varepsilon_r$ = 4 nominal) was obtained on a thin silicon oxide ($Si0_2$) film (see appendix).

Besides that we also compared it with another procedure followed previously in our group, the constant height, resulting in exactly same values. This result enables us to choose either one of the techniques at our convenience.