Calibrated complex impedance of CHO cells and *E. coli* bacteria at GHz frequencies using scanning microwave microscopy

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Received 16 November 2015, revised 23 December 2015
Accepted for publication 28 January 2016
Published 22 February 2016

Abstract

The application of scanning microwave microscopy (SMM) to extract calibrated electrical properties of cells and bacteria in air is presented. From the *S*₁₁ images, after calibration, complex impedance and admittance images of Chinese hamster ovary cells and *E. coli* bacteria deposited on a silicon substrate have been obtained. The broadband capabilities of SMM have been used to characterize the bio-samples between 2 GHz and 20 GHz. The resulting calibrated cell and bacteria admittance at 19 GHz were $Y_{\text{cell}} = 185 \mu S + j285 \mu S$ and $Y_{\text{bacteria}} = 3 \mu S + j20 \mu S$, respectively. A combined circuitry-3D finite element method EMPro model has been developed and used to investigate the frequency response of the complex impedance and admittance of the SMM setup. Based on a proposed parallel resistance–capacitance model, the equivalent conductance and parallel capacitance of the cells and bacteria were obtained from the SMM images. The influence of humidity and frequency on the cell conductance was experimentally studied. To compare the cell conductance with bulk water properties, we measured the imaginary part of the bulk water loss with a dielectric probe kit in the same frequency range resulting in a high level of agreement.

Keywords: scanning microwave microscopy, complex impedance, permittivity, cells, bacteria

(Some figures may appear in colour only in the online journal)

1. Introduction

Scanning microwave microscopy (SMM) is an electrical characterisation technique used to extract material properties at high frequencies with nanometre accuracy [1–5]. SMM interfaces two well-known measurement tools, the atomic force microscope (AFM) for materials characterisation and the vector network analyser (VNA) for high-frequency signal measurements. The AFM allows for nanometre lateral resolution imaging, and the VNA provides high precision impedance and admittance measurements at broadband frequencies from MHz to GHz. In reflection mode SMM, the VNA detects the properties of the sample under test (SUT) by probing the scattering reflection parameter, which represents the ratio of the reflected and incident microwave signals, at the tip/sample contact point. The conductive tip, attached to a solid metal cantilever, acts both as a nanometre-scale AFM probe and as a GHz emitter–receiver antenna. Depending on the sample impedance, part of the microwave signal is reflected and measured by the VNA.

The complex scattering parameter measured by the VNA includes, along the SUT impedance information, effects from the microwave cables and connectors. The raw SMM signal is
therefore de-embedded and converted to meaningful physical quantities, such as complex impedance, capacitance, and conductance, by means of a calibration procedure. Different approaches have been proposed as workflows for providing a reliable calibration of the system [6, 7]. For the samples studied in this paper [7], the most practical calibration method, as it allows performing an immediate in situ calibration of the measurement, without the use of a calibration sample. The calibration is thereby performed by interfacing SMM and electrostatic force microscopy (EFM), based on the electrostatic force–capacitance relation presented in [8].

Significant advances in SMM measurement and calibration workflows were achieved by studying well-defined semiconductor devices. Originating from the requirement of the semiconductor industry to identify failures and leakages in electronic devices with nanometre precision and low impedance values [9], the SMM has since proved to be a powerful tool also for materials science applications [10]. Moreover, the capability of sub-surface imaging of buried nanoscale structures has been demonstrated [11, 12]. Recently, the high frequency characterisation of biological materials has attracted considerable interests [13–18], as SMM represents a convenient non-invasive evanescent imaging technique that complements AFM. In the following, we present calibrated capacitance and conductance images of individual Chinese hamster ovary (CHO) cells and E. coli bacteria measured in air at around 20 GHz using the SMM.

2. Materials and methods

2.1. SMM setup

The SMM is composed of the 5600LS AFM connected to an E8362B (10 MHz–20 GHz) VNA (both from Keysight Technologies, Santa Rosa, CA, USA). The conductive solid Pt AFM probes are from Rocky Mountain Nanotechnology (Salt Lake City, UT, USA) with a nominal tip radius of 80 nm and spring constant of 18 N m⁻¹. SMM enables different measurement modes, allowing the user to customise the level of interaction between the probe and the sample, varying from non-contact, to intermittent contact or tapping mode, and contact mode. In this work, we employed contact mode (cell images in figures 5 and 6) and tapping mode (bacteria images in figure 4) at the tips’ nominal tapping resonance frequency, 20 kHz. Occasionally, due to mechanical imaging forces, single bacteria were moved to different positions in subsequent AFM scans.

In SMM, the tip scans the sample surface and in every measurement point of the scan, the S11 scattering parameter is acquired by the VNA (see figure 1). The high-frequency signal travels from the VNA to the conductive probe, which is in contact to the sample or tapping the sample. Depending on its electrical properties (i.e. the impedance of the sample relative to the probe impedance), the sample partly absorbs or reflects the high-frequency wave. The reflected wave travels through the transmission line back to the VNA, where it is compared with a copy of the incident wave. The numerical value of S11 scattering parameter measured in dB is obtained as 20 · log₁₀ \( \frac{V_{\text{ref}}}{V_{\text{inc}}} \) or 10 · log₁₀ \( \frac{P_{\text{ref}}}{P_{\text{inc}}} \), where \( V_{\text{ref}} \) is the reflected wave voltage, \( V_{\text{inc}} \) is the incident wave voltage, and \( P_{\text{ref}} \) and \( P_{\text{inc}} \) are the respective power values. The maximum possible value of S11 is 0 dB, if 100% of the wave is reflected from the sample, and the minimum S11 is achieved when the full wave is absorbed by the load, i.e. an impedance matching condition is obtained. The characteristic impedance of the VNA is 50 Ω. The impedance of the nanometre scale tip at GHz frequencies is in the range of several kΩ [19]. An impedance matching network is inserted along the transmission line in order to adapt the system impedance to that of the probe, hence allowing the probe to be sensitive to minute changes in the sample impedance. It consists of a 50 Ω shunt resistor and a half-wavelength resonator with an effective length of 9 cm, and an inner dielectric relative permittivity of 3.72 (figure 2(a)). The resonator acts as a pass band filter for the harmonics of 1 GHz, with a 3 dB bandwidth of ~50 MHz, hence creating a pattern in the S11 frequency response, as seen in figure 2(b). The SMM measurements are performed in the proximity of the minima, where the resonator creates an evanescent wave. A 30 dB amplification of the reflected signal, along with an improvement of the system’s noise response has been provided by the dopant profiling measurement module (DPMM; Keysight Technologies, Santa Rosa, CA, USA), which was employed for all the measurements presented. The incident microwave power was set to ~9 dBm.

2.2. ADS and EMPro modelling

The frequency behaviour of the SMM has been investigated using advanced design system (ADS; Keysight Technologies)
Firstly, the standard components have been modelled, i.e. the 50 Ω power source as the VNA, the coaxial cable, the 50 Ω shunt resistor and the resonator cable. The load of the network (i.e. the tip/cell structure) has been included into the model using 3D electromagnetic finite element method (FEM) and simulated with electromagnetic professional (EMPro; Keysight Technologies). For the EMPPro model, the cell was modelled with a 30 μm diameter placed on top of a three-layered stack substrate (see figure 3). The cantilever is 100 μm wide and 300 μm long. The tip of the probe is modelled as a frustum with an upper diameter of 80 μm and a nanoscopic sphere as contact object at the bottom. The radius of the very end of the probe tip is set to 100 nm. Both the cantilever and the tip are modelled as perfect electrical conductors (PEC) because they are made of highly conductive platinum. The three-layered stack substrate

**Figure 2.** SMM frequency response and circuit modelling. (a) ADS equivalent circuit model of SMM impedance matching network and parallel RC load describing the tip/sample interaction. (b) Smith chart showing the influence of impedance matching network on $S_{11}$ response. The red points on the outer ring are simulated without impedance matching network, while the blue points in the origin include the matching network. Both sets of values represent the variation of $S_{11}$ from $\infty$ to 0 (i.e. from open to short circuit). (c) SMM frequency sweep curves showing $S_{11}$ response over three frequency ranges, obtained experimentally (blue curves) and simulated with ADS and EMPro (red curves).

**Figure 3.** 3D electromagnetic model of AFM tip in contact with a cell using EMPro. (a) Geometric 3D model overview of cantilever and tip in contact to a cell located on top of SiO₂ pad and Si²⁺ substrate. The electric port exciting the metallic cantilever is shown as an arrow. (b) Finite element modelling (FEM) meshing of tip and cell, including membrane, spherical nucleus, vacuoles, and inner liquid. (c) and (d) Distribution of electric field over cell membrane and penetration depth inside the cell with tip located above cell nucleus (c) and above the vacuoles (d). Calculated complex admittance values are given for 10 GHz.
is composed of thin SiO₂ (thickness 50 nm; relative permittivity 3.9), doped Si (10 μm thick; dopant density 10¹⁷ atoms cm⁻³); conductivity 500 S m⁻¹), and the bottom is a thin PEC layer representing the sample holder, which provides the return path for the MW current.

2.3. Complex impedance calibration

A recently developed workflow was used to calibrate the SMM [7]. In short, tip–sample approach curves are acquired simultaneously at low kHz frequency (EFM-mode) and GHz frequency (SMM-mode). The magnitude and phase of the S₁₁ parameters and the EFM signals are acquired during tip–sample approach. The capacitance is calculated from the electrostatic force using \( \frac{dC}{dz} = 4F_{es,2\omega}/V_0^2 \), where the applied voltage is \( V(t) = V_0 \sin(\omega t) \), and \( F_{es,2\omega} \) is the electrostatic force. This represents the variation of capacitance with height, which is the z-distance between the tip and the surface. From this, by integration, the capacitance value is extracted. Assuming that the impedance variation is exclusively capacitive (i.e. that the substrate is highly conductive or lossless dielectric), the three error parameters \( e_0, e_1, e_2 \) are calculated from \( S_{11,m} = e_0 + e_1S_{11,a}/(1 - e_1S_{11,a}) \), where 
\[
S_{11,a} = \frac{(Z_{tip} - Z_{sub})}{(Z_{tip} + Z_{sub})}.
\]
In order to be compliant with the requirement of a highly conductive or lossless dielectric substrate, the substrate of choice has been highly B doped Si substrate. On the doped Si substrate, also a pattern of 20 nm high SiO₂ pillars is present, which is however not relevant for the calibration. The oxide pillars have been used to verify the impedance calibration as the oxide should show a contrast in the capacitance image but no contrast in the conductance image. The advantage of this calibration workflow is that no specific calibration sample is required and that the calibration can be performed on the bare conductive substrate.

The tip radius has been extracted by fitting the capacitance values obtained experimentally from approach curves, using a tip/sample geometrical model. The analytical model of the capacitance relies on the tip model proposed in [21, 22], and [7], where three summed capacitance components provide the total capacitance between the tip and the substrate, namely the contributions from tip apex, the tip cone and the stray capacitance. The apex part is expressed as 
\[
C_{apex}(z, \varepsilon_r) = 2\pi \varepsilon_0 R \ln \left( 1 + \frac{R^2 (1 - \sin \theta)}{z^2 + h^2/\varepsilon_r} \right),
\]
where \( \varepsilon_r \) is the relative dielectric constant, \( R \) is the tip apex radius, \( \theta \) is the half cone angle, \( h \) is the thin film thickness, and \( z \) is the distance between the tip and the substrate. The capacitance induced by the tip cone is obtained from
\[
C_{cone}(z, \varepsilon_r) = \frac{-2\pi \varepsilon_0}{\ln \tan(\theta/2)} \left[ \frac{z + \varepsilon_r}{z + \varepsilon_r + R(1 - \sin \theta)} \right] 
\times \ln \left[ \frac{z + \varepsilon_r}{z + \varepsilon_r + R(1 - \sin \theta)} + \frac{R \cos \theta}{\sin \theta} \right] 
\]
The stray component of the capacitance is calculated from \( C_{stray} = C_{stray}z + c \), where \( C_{stray} \) is the linear part of the capacitance variation, taken from the calibration curve and \( c \) is an offset constant. The total analytical model capacitance is 
\[
C_{total} = C_{apex} + C_{cone} + C_{stray},
\] which has been compared with the experimentally obtained capacitance in order to obtain the tip radius.

2.4. Sample preparation

Prior to cells deposition, the Si substrate was cleaned with isopropanol and acetone. Then, an adapted method of the protocol for cell deposition on graphite and glass shown in [20] was applied to the silicon substrate. In short, the flask containing the cells was washed three times with 4 ml phosphate-buffered saline (PBS), then 1 ml Trypsin added into the flask for 1–3 min. 2 ml of medium (1:1 mixture of DMEM and Ham’s F12, with 10% FBS and 0.1% gentamycin) was then transferred over 1 ml of cell suspension into a Falcon tube. After centrifuging and removing the liquid, 2 ml medium was added to the cell pellet. The above cell solution was used either directly, or diluted 1:3 or 1:10. 100 μl cell solution was placed on the cleaned Si wafer. The substrate with the cell solution was incubated for at least 3 h in the cell culture incubator at 37 °C and 5% CO₂. After removing the solution from the substrate, 100 μl 4% formaldehyde (in HBSS—Hanks’ balanced salt solution, with Ca²⁺) was given on the substrate with cells and the substrate was washed with HBSS for three times, then with Millipore water for other three times. Finally, the sample was dried with a gentle nitrogen flow.

2.5. Tip radius extraction

The tip radius has been extracted by fitting the capacitance values obtained experimentally from approach curves, using a tip/sample geometrical model. The analytical model of the capacitance relies on the tip model proposed in [21, 22], and [7], where three summed capacitance components provide the total capacitance between the tip and the substrate, namely the contributions from tip apex, the tip cone and the stray capacitance. The apex part is expressed as 
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C_{apex}(z, \varepsilon_r) = 2\pi \varepsilon_0 R \ln \left( 1 + \frac{R^2 (1 - \sin \theta)}{z^2 + h^2/\varepsilon_r} \right),
\]
where \( \varepsilon_r \) is the relative dielectric constant, \( R \) is the tip apex radius, \( \theta \) is the half cone angle, \( h \) is the thin film thickness, and \( z \) is the distance between the tip and the substrate. The capacitance induced by the tip cone is obtained from
\[
C_{cone}(z, \varepsilon_r) = \frac{-2\pi \varepsilon_0}{\ln \tan(\theta/2)} \left[ \frac{z + \varepsilon_r}{z + \varepsilon_r + R(1 - \sin \theta)} \right] 
\times \ln \left[ \frac{z + \varepsilon_r}{z + \varepsilon_r + R(1 - \sin \theta)} + \frac{R \cos \theta}{\sin \theta} \right] 
\]
The stray component of the capacitance is calculated from \( C_{stray} = C_{stray}z + c \), where \( C_{stray} \) is the linear part of the capacitance variation, taken from the calibration curve and \( c \) is an offset constant. The total analytical model capacitance is 
\[
C_{total} = C_{apex} + C_{cone} + C_{stray},
\] which has been compared with the experimentally obtained capacitance in order to obtain the tip radius.

2.6. Topography crosstalk removal

Based on the cantilever–substrate stray capacitance, the topography of the sample influences the absolute values of the tip–sample capacitance. In order to mitigate this, a linear topography crosstalk removal procedure has been applied during data post-processing. The procedure aims to minimise the effect of the topography-induced capacitance. It does this by subtracting, pixel by pixel over the scanned image, the non-local capacitance from the raw calibrated capacitance. The non-local capacitance represents the product of the multiplication of the stray capacitance \( c_{stray} \) in aF nm⁻¹ (attoFarrad/nanometre) and the topography (in nm). The
stray capacitance is extracted from the linear part of the SMM curve taken for calibration.

2.7. Dielectric probe kit measurements

The dielectric measurements presented in figure 6(c) have been performed using the N1501A dielectric probe kit connected to N9918A FieldFox handheld microwave analyzer, 26.5 GHz (both from Keysight Technologies, Santa Rosa, CA, USA). The complex permittivity of Milli-Q water (Millipore Corporation, MA, US), 0.1 M PBS (GE Healthcare, UK) solution and HBSS (GE Healthcare, UK) has been acquired over the 1–20 GHz frequency range, at room temperature 25 °C. The observed values are in agreement with the Debye relaxation model and with the corresponding literature [23, 24].

3. Results and discussion

For quantitative SMM impedance imaging, we established a tip–sample circuitry model including a parallel resistance–capacitance (RC) circuit between the tip, sample, and substrate (figure 1). Additionally, the parasitic capacitances formed between the probe shank and the substrate, $C_{\text{con}}$, as well as $C_{\text{stray}}$, formed between cantilever chip and substrate, have been included in the model to account for stray capacitance effects. This circuitry model was quantitatively studied in ADS in the frequency range 1–20 GHz including the effects of cables and resonators from the SMM hardware (figure 2(a)). The modelled reflection $S_{11}$ sweep curve is shown in figure 2(b) at various frequency ranges and is compared with the experimentally acquired SMM sweep. In the full 20 GHz sweep the 50 $\Omega$ shunt resistor, along with the half wavelength resonator induce the notch pattern every 900 MHz. In the notches, the impedance value of the tip–sample system matches the intrinsic impedance of the VNA, 50 $\Omega$, and highly sensitive SMM measurements can be performed. The narrower the frequency sweep range, the better is the fitting between experiment and simulation. At the level of a single notch, the experimental data agrees well with the ADS model. However, the remaining disagreement between model and experimental frequency sweep indicates that not all features in the transmission part have been captured by the model. Figure 2(c) shows the modelling results in a Smith Chart typically used for high frequency network analysis, in which both the scattering parameter $S_{11}$ and the impedance $Z$ are plotted. The variation of $S_{11}$ between 2.3 GHz and 2.8 GHz, as shown in figure 2(b), is replicated on the Smith Chart with the minimum of the notch corresponding to the chart’s origin. Additionally, the impedance variation is shown with and without the impedance matching network. It can be observed that the impedance matching network shifts the measured impedance values toward the origin of the Smith Chart, i.e. where the sensitivity of the system reaches the maximum.

Figure 3 shows the microwave interaction at the tip/sample contact point using the FEM tool in EMPro [25]. A 3D cell-like structure has been implemented in computer-aided design (CAD; figure 3(a)) including the cell nucleus, three vacuoles, and a 10 nm thick membrane, improving a model recently presented in [26]. The simulated values of admittance were obtained at 10 GHz and at different positions over the cell, in order to investigate how different cell compartments influence the electric field distribution. Different dielectric values were used for the individual cell compartments including the lipid bilayer of the cell membrane ($\varepsilon_r = 3.2$) [27], the cell nucleus with DNA ($\varepsilon_r = 8$) [28] and a protein vacuole ($\varepsilon_r = 3$) [29]. Depending on the cell and tip geometry, the simulated tip–cell complex admittance was determined as roughly $Y \approx 110 + j250 \mu\text{s}$, which translates to conductance values of 80–110 $\mu\text{s}$ and capacitance values of 2000–4000 aF.

Based on the frequency response modelling the operating SMM frequency was adjusted to achieve highest sensitivity and SMM images of cells and bacteria were acquired at fixed frequencies between 1 and 20 GHz. In figure 4, SMM images of E. coli bacteria acquired in air are shown at 19.9 GHz frequency. The images have been acquired in tapping mode, which, compared to contact mode, is a gentler imaging mode with intermittent contact. Standard values of bacteria length (2–2.5 $\mu$m) and height (200–250 nm) have been obtained in the topography images (figures 4(a), (b), and (e)). Using the recently developed black-box calibration procedure [7], the complex $S_{11}$ images are converted to impedance images. For this, tip–sample approach curves were acquired at the same microwave frequency on the Si substrate and a standard VNA calibration model using three complex $\varepsilon$-parameters was used to transfer $S_{11}$ into complex impedance $Z$ (see materials and methods). After calibration, the topography crosstalk between the topography image and the capacitance image was removed, allowing the subtraction of the cantilever based stray capacitance from the local AFM tip capacitance. The stray capacitance is thereby extracted from the linear part of the $S_{11}$ approach curve, and the topography-influenced values are subtracted pixel-by-pixel from the raw capacitance values. The resulting capacitance values prove that the capacitance contrast is induced by the different dielectric properties of bacteria, SiO$_2$, and Si$^{++}$. In the capacitance images, the SMM contrast between bacteria, SiO$_2$ pillars, and Si$^{++}$ substrate, varies between 10 and 150 aF (figures 4(c) and (f)), depending on the different radii of the tips used for scanning. The tip radius has been determined experimentally including a proper tip-geometry model (see materials and methods), ranging thereby from 250 nm to 900 nm. The error of the capacitance and conductance calibration is $\sim$5% [7]. The oxide pillars show a signal only in the capacitance image and not in the conductance image, as expected from a pure dielectric material, proving the validity of the complex impedance calibration. No significant conductance contrast is obtained between the bacteria, the SiO$_2$ pillars, and the Si$^{++}$ substrate, as expected from gram-negative type bacteria as E. coli, with a water repellent membrane, hence they are non-conductive entities, with no significant water layer adsorbed (figures 4(d) and (g)) [30]. No topography crosstalk is obtained in the
is proportional to the conductance image, corroborating the specificity of the measurements.

Figures 5(a)–(c) shows the raw topography, $S_{11}$ amplitude, and phase images of single CHO cells acquired in air. Due to their large dimensions, they can be easily spotted on the silicon substrate and individually scanned in contact mode. The topography images show a diameter of roughly 30 $\mu$m and a height of more than 1 $\mu$m. The raw SMM images, acquired at 18.9 GHz, show the cell structure with high signal levels of 2 dB in $S_{11}$ amplitude and 7° in $S_{11}$ phase with respect to the substrate. The same complex impedance calibration workflow was applied as described for the bacteria. The obtained capacitance image (figure 5(d)) shows a contrast of 2400 aF (attoFarad) between the cell and the substrate. The negative values over the cell are accountable to the reference capacitance formed between the tip and the thin ($\sim$1 nm thick) native SiO$_2$ layer deposited on top of Si$^{++}$. The crosstalk between the raw capacitance values and the topography has been cancelled as per materials and methods. The obtained capacitance variation is induced by cell dielectric properties that are different from the Si substrate dielectric properties. The conductance image in figure 5(e) shows a higher conductance ($\sim$200 $\mu$S) of the cell compared to the substrate. Figure 5(f) shows the calibrated conductance image of the same cell acquired at a lower frequency of 2 GHz and at the same ambient humidity (30% relative humidity, RH). A lower conductance of the cell is thereby obtained at lower frequency, which is also a typical behaviour of bulk water [29]. We therefore attribute the conductance properties of the cell at different frequencies to the presence of water, either within the cell or adsorbed to the cell membrane. While the frequency behaviour of the cell is consistent with the variation of the imaginary part of the complex permittivity of bulk water, a more detailed investigation is shown in the following.

A set of measurements with controlled variation of the ambient humidity has been performed on CHO cells (figure 6). The values of the humidity have been varied, from low humidity (figures 6(a), 2% RH, obtained in dry nitrogen environment) to high humidity (figure 6(b), 60% RH), while the SMM frequency was set to 19.9 GHz in both cases. The calibrated conductance values obtained at low humidity are in the order of 10–20 $\mu$S, whereas the values obtained at high humidity vary between 30 and 200 $\mu$S, which can be explained with the variation of the conductivity based on the water content in the cells. Figure 6(c) shows the variation of the complex permittivity of different aqueous solutions (pure water, PBS, and HBSS) with respect to frequency ranging from 1 to 20 GHz, recorded with the dielectric probe kit, at ambient temperature 25 °C. The choice of the three solutions should match the cell buffer solutions. The real part $\varepsilon'$ of the water dielectric constant thereby decreases from $\varepsilon'_{\text{water}} = 78$ at 2 GHz to $\varepsilon'_{\text{water}} = 40$ at 20 GHz. The imaginary part of the water relative permittivity $\varepsilon''$ is proportional to the conductivity $\sigma$, based on $\sigma = 2\pi f\varepsilon''\varepsilon_0$, where $\varepsilon_0$ is the permittivity of free space, and $f$ is the frequency. The dielectric probe measurements reveal a low value of the imaginary part of the relative permittivity ($\varepsilon''_{\text{water}} < 10$, $\sigma_{\text{water}} < 1$ S m$^{-1}$) at low frequency (2 GHz) and a high value ($\varepsilon''_{\text{water}} = 35$, $\sigma_{\text{water}} = 40$ S m$^{-1}$) at high frequency (20 GHz). The SMM cell conductivity can now be compared to the water
Figure 5. Calibrated complex impedance images of a CHO cell in air at 18.9 GHz, at ∼30% RH. (a) SMM topography image taken in contact mode with a scan size of 37 μm × 37 μm. Typical cell height is 1 μm. (b) S11 amplitude image in dB. (c) S11 phase image in degrees. (d) Calibrated capacitance image. (e) Calibrated conductance image. (f) Calibrated conductance of the same CHO cell, imaged at 2 GHz. The three corresponding cross-section profiles are shown.

Figure 6. Calibrated cell conductance images measured at different ambient humidities at 19.9 GHz. It compares low (2% RH; (a)) and high (60% RH; (b)) humidity of calibrated conductance images. Inset shows cell topography. (c) Real and imaginary parts of water complex permittivity, 0.1 M PBS and HBSS buffers as a function of frequency, over the range 1–20 GHz, experimentally obtained using dielectric probe kit.
The SMM conductivity at 20 GHz. The SMM conductivity $\sigma$ can be obtained from the conductance $G$ in a simple first order approximation from the volume of the cell in contact with the SMM tip, and applying the relation $G = 1/A$, where $A = \pi R_{\text{apex}}^2$ ($R_{\text{apex}} = 900 \text{ nm}$) is the contact area and $l = 1.25 \mu\text{m}$ is the cell height obtained from the topography image. The effective conductivity obtained from the SMM cell conductance, at $f = 20 \text{ GHz}$, is $\sigma_{\text{cell}} = 18 \text{ S m}^{-1}$ and effective $\varepsilon_{\text{eff.cell}} = 15$, which is in the range of the bulk water permittivity. Accordingly, the cell conductivity behaviour at different frequencies and ambient humidity is similar to bulk water properties, corroborating the hypothesis that water is adsorbed on the cells’ surface and that it is responsible for the MW energy dissipation over the cells.

4. Conclusion

We show impedance images of bacteria and cells acquired at microwave frequencies around 20 GHz. On the basis of transmission line modelling, measurement parameters were carefully optimised to obtain good signal-to-noise SMM images. The tip/sample interaction is modelled using 3D EMPro and the admittance values (admittance $Y = 1/\text{impedance } Z$) were calculated in the frequency range of $1–20 \text{ GHz}$. The 3D modelling allows studying the influence of the tip geometry on the complex impedance values. In addition, the effect of different substrates and layer thicknesses on the system impedance can be considered. From calibrated SMM measurements we obtained the complex admittance (complex impedance) of bacteria and cells at $\sim 19 \text{ GHz}$, resulting in roughly $Y_{\text{bacteria}} = 5 \mu\text{s} + j20 \mu\text{s}$ ($Z_{\text{bacteria}} = 7 \text{k} \Omega - j50 \text{k} \Omega$), and $Y_{\text{cell}} = 185 \mu\text{s} + j285 \mu\text{s}$ ($Z_{\text{cell}} = 1.6 \text{k} \Omega - j2.5 \text{k} \Omega$), respectively. The gram-negative $E. coli$ bacteria show almost no conductance at high frequencies while CHO cells exhibit conductance values of up to $200 \mu\text{s}$. The impedance calibration workflow based on the approach curve analysis has an uncertainty of $5\%$. The cell conductivity was compared to the bulk water conductivity. The broadband complex permittivity values of water and buffer solutions were acquired with the dielectric probe kit. The SMM cell conductivity was determined to $\sigma_{\text{cell}} = 18 \text{ S m}^{-1}$ ($\varepsilon''_{\text{cell}} = 15$) which shows a high level of agreement to the measured bulk water conductivity at $20 \text{ GHz}$ ($\sigma_{\text{water}} = 40 \text{ S m}^{-1}$; $\varepsilon''_{\text{water}} = 35$). The tan $\delta$ value describing the ratio of imaginary and real part of the permittivity was measured to be $\tan \delta_{\text{water}} = 0.875$ for bulk water at $20 \text{ GHz}$. The high value of tan $\delta$ is typical for lossy materials that have significant energy dissipation at the corresponding frequency. The energy loss is particularly high at frequencies around $20 \text{ GHz}$ where water has a maximum in $\varepsilon''$ due to the dipolar polarisation [23]. At lower frequency e.g. $2 \text{ GHz}$ the tan $\delta_{\text{water}}$ is only $0.01$ and $\sigma_{\text{cell}} = 4 \text{ S m}^{-1}$. Hence, a similar frequency dependence of the imaginary part of permittivity and conductivity has been obtained for cells and water, leading to the conclusion that the water plays a major role in the loss behaviour of cells.

In summary, we present a broadband analysis of calibrated nanoscale impedance measurements using the SMM.

The complex permittivity of biological materials, including conductivity has been measured and found to be strongly related to bulk water properties.

Acknowledgments

The authors would like to thank Gerald Kada, Giulio Maria Campagnaro, Matthias Fenner, and Hassan Tanbakuchi from Keysight Technologies for the help and expertise provided throughout the project. This work has been supported by EU-FP7 (PEOPLE-2012-ITN-317116, NANOMICROWAVE), Bio-SMM FFG (Project No. 846532), and FWF Project P 28018-B27.

References

[1] Imtiaz A et al. 2014 Near-field scanning microwave microscopy IEEE Microw. Mag. 15 52–64
[2] Anlage S M et al. 2007 Principles of near-field microwave microscopy Scanning Probe Microscopy: Electrical and Electromechanical Phenomena at the Nanoscale vol 1 ed S V Kalinin and A Grueveman (New York: Springer) pp 215–53 ISBN: 978-0-387-28667-9
[3] Talanov V V et al. 2009 Near-field scanning microwave microscope for interline capacitance characterization of nanoelectronics interconnect IEEE Trans. MTT 57 1224–9
[4] Gregory A et al. 2012 Spatially resolved electrical characterisation of graphene layers by an evanescent field microwave microscope Physica E 56 431–4
[5] Vlahacos C P et al. 1996 Near-field scanning microwave microscope with 100 μm resolution Appl. Phys. Lett. 69 3272–4
[6] Gao C et al. 1997 High spatial resolution quantitative microwave impedance microscopy by a scanning tip microwave near-field microscope Appl. Phys. Lett. 71 1872–4
[7] Gramse G et al. 2014 Calibrated complex impedance and permittivity measurements with SMM Nanotechnology 25 8
[8] Girard P et al. 2001 Electrostatic force microscopy: principles and some applications to semiconductors Nanotechnology 12 485–90
[9] Schweinböck T et al. 2014 Quantitative scanning microwave microscopy: a calibration flow Microelectron. Reliab. 54 2070–4
[10] Hoffmann J et al. 2015 Measuring low loss dielectric substrates with scanning probe microscopes App. Phys. Lett. 105 013102
[11] Plassard C et al. 2011 Detection of defects buried in metallic samples by scanning microwave microscopy Phys. Rev. B 83 121409
[12] Gramse G et al. 2015 Quantitative sub-surface and non-contact imaging using scanning microwave microscopy Nanotechnology 26 135701
[13] Oh Y J et al. 2011 High-frequency electromagnetic dynamics properties of THP1 cells using scanning microwave microscopy Ultramicroscopy 111 1625–9
[14] Farina M et al. 2012 Tomographic effects of near-field microwave microscopy in the investigation of muscle cells interacting with multi-walled carbon nanotubes Appl. Phys. Lett. 101 203101
[15] Park J et al. 2005 Observation of biological samples using a scanning microwave microscope Ultramicroscopy 102 101–6
Lee K et al 2013 Label-free DNA microarray bioassays using a near-field scanning microwave microscope Biosens. Bioelectron. 42 326–31
Tabib-Azar M et al 1999 Evanescent microwaves: a novel super-resolution noncontact nondestructive imaging technique for biological applications IEEE Trans. Instrum. Meas. 48 1111–6
Haddadi K et al 2015 Sensing of liquid droplets with a scanning near-field microwave microscope Sensors Actuators A 230 170–4
Happy H et al 2014 Measurement techniques for RF nanoelectronic devices IEEE Microw. Mag. 1527–3342 30–9
Duman M et al 2010 Improved localization of cellular membrane receptors using combined fluorescence microscopy and simultaneous topography and recognition imaging Nanotechnology 21 115504
Hudlet A et al 1998 Evaluation of the capacitive force between an atomic force microscopy tip and a metallic surface Eur. Phys. J. B 2 5–10
Gomila G et al 2014 Finite-size effects and analytical modeling of electrostatic force microscopy applied to dielectric films Nanotechnology 25 255702
Gabriel C 2007 Dielectric properties of biological materials Bioengineering and Biophysical Aspects of Electromagnetic Fields 3rd edn ed F S Barnes and B Greenebaum (Boca Raton, FL: CRC Press) pp 52–94 ISBN: 978-0-8493-9539-4
Stuchly M A et al 1980 Dielectric properties of biological substances—tabulated J. Microw. Power 15 19–26
Kasper M et al 2013 Electromagnetic simulations at the nanoscale: EMPro modeling and comparison to SMM experiments (http://literature.cdn.keysight.com/litweb/pdf/5991-2907EN.pdf)
Tuca S S et al 2015 Single E. coli bacteria imaged at 20 GHz frequency using the scanning microwave microscope (SMM) Microsc. Anal. 29 9–12
Gramse G et al 2013 Nanoscale measurement of the dielectric constant of supported lipid bilayers in aqueous solutions with electrostatic force microscopy Biophys. J. 104 1257–62
Cuervo A et al 2014 Direct measurement of the dielectric polarization properties of DNA Proc. Natl Acad. Sci. 111 E3624–30
Kukic P et al 2013 Protein dielectric constants determined from NMR chemical shift perturbations J. Am. Chem. Soc. 135 16968–76
Esteban-Ferrer D et al 2014 Electric polarization properties of single bacteria measured with electrostatic force microscopy ACS Nano 8 8843–8449