Electrical properties of Jurkat cells: an inverted scanning microwave microscope study

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Abstract—Near-field Scanning Microwave Microscopy (SMM) makes use of a high frequency signal to image and characterize electrical properties of samples. Recently, a new SMM setup was developed, the so called inverted-SMM (iSMM), whose biocompatibility allows its application to sample of biological interest. The experimental arrangement of the iSMM combines an Atomic Force Microscope (AFM), a Vector Network Analyser (VNA) and a slot line as a sample holder.

In this work, a calibration protocol for reflection mode measurements is applied to the imaging of biological samples, in particular Jurkat cells. The complex local admittance of a single cell is extracted and the dielectric constant is estimated to be around 2.6 ± 0.3. Thus, the first quantitative characterization of iSMM operating in reflection mode is reported, as well as the first electrical characterization of Jurkat cells by this tool.

Keywords— Scanning Microwave Microscopy, iSMM, Jurkat Cells, Calibration.

I. INTRODUCTION

Scanning probe microscopy (SPM) has improved our understanding of surfaces and materials at sub-nanometric scale. In this class of devices, a probe acquires variation of some physical/chemical parameters arising from the short-range interactions between the probe itself and the sample surface [1]. Recently, a new class of techniques started to evolve, namely the near-field scanning microwave microscopy (SMM). SMM is commonly coupled to other scanning probe microscopy techniques, such as the atomic force microscopy (AFM) or the scanning tunneling microscopy (STM).

SMM evaluates the probe-sample interactions using an evanescent/near field, which decays exponentially from the probe (source) [2]. The main concept of the standard SMM is that SPM probe acts also as an “antenna” to transmit and to receive the illuminating microwave signal. The microwaves reach the sample (incident signal) and then are collected back (reflected signal) after the interaction with the sample; the microwave signal is generated and recorded by a vector network analyser (VNA).

Imtiaz and Anlage [3] combined a microwave signal and a standard tunneling microscope with its feedback circuit to keep the probe at a fixed distance from the sample. In a reflectometric measurement, the microwave signal allowed a direct non-invasive mapping of complex impedance at nanometric resolution, whose manipulation can be further used to access other electrical properties of the sample. On the other hand, some authors used AFM to drive the feedback circuits, to access other electrical properties of the sample. On the other hand, some authors used AFM to drive the feedback circuits, while using the AFM conductive tip as microwave probe. Tabib-Azar and Wang [4] successfully developed a similar system, where the tip was controlled by an AFM. Following this strategy, Han Tanbakuchi and other researchers in Agilent (now Keysight) introduced a scanning impedance microscope, exploiting a VNA and an atomic force controller [5-7]. The device takes advantage of the very large dynamics of the currently available VNA and makes possible accurate quantitative measurements of the complex impedance. An excellent state-of-the-art description of SMM technology is reported in a review paper by Imtiaz and coworkers [8].

Generally, the above described systems are intrinsically "narrow-band", i.e. limited frequency range is used and generally a resonator or an interferometer is introduced to increase sensitivity; however, having a broadband system opens the possibility to perform a local microwave spectroscopy, especially attractive for biological applications. In fact, many cellular structures have polar properties, giving rise to phonon excitation when irradiated by time-varying electromagnetic field. As a matter of fact, a patent dated September 2009 by Sun et al. [9] exploits the microwave resonant absorption of viruses through dipolar coupling to viral acoustic modes, introducing a sensor to identify different viruses. Moreover, near field microwave microscopy has demonstrated capabilities in measuring dielectric permittivity and conductivity on a local basis [10-12].

Recently, the use of SPM-based SMM has gained attention in biological and biomedical fields [13-15] thanks to the capability of this technique to measure parameters such as the dielectric constant of lipid bilayers [16], the electric permittivity of single bacterial cells [11] and the complex impedance of epithelial cell line CHO cells and Escherichia coli [12]. However until now, the applications of SMM are mainly restricted to surface science and semiconductor materials while...
the biological uses are extremely limited [17]. The improvement of SMM technique towards a biocompatible approach could open a new wide range of applications in medical and biological field. For instance, the study of electrical properties variations of cells and sub-cellular structures in presence of diseases or correlated with physiological changes such as during aging can be addressed with this technique.

In this work, we discuss the characterization of Jurkat cells (human lymphocyte cells) by using the so-called inverted scanning microwave microscope (iSMM) introduced few months ago [18]. Consequently, iSMM is here employed and tested in the context of non-invasive and biocompatible microscopy for quantitative sample characterization. In iSMM system, the conductive scanning probe is always grounded, and the microwave signal is injected through a transmission line that becomes, in this way, the sample holder. The input and output of the transmission line are connected to a VNA, so that both reflected and transmitted signals (S11 and S21, respectively) can be measured [18-19]. In the following sections, S11 data are obtained and calibrated allowing the evaluation of local complex admittance of a single Jurkat cell. Dielectric constant of Jurkat cell is then obtained with an existing analytical model. Thus, this procedure allowed to obtain the first quantitative electrical characterization of a sample from iSMM operating in reflection mode.

II. EXPERIMENTAL SETUP AND SAMPLE PREPARATION

A. Experimental setup

The iSMM experimental setup includes a Keysight Technologies 7500 AFM and a sample holder with an integrated gold slot line. The schematic of the microscope is depicted in Fig. 1. The microwave power is injected through the slot line while the AFM metal probe is grounded. The AFM probe is used only to electrically perturb the slot line while scanning the sample. This allows to convert easily an AFM or other SPMs into a two ports SMM.

![Fig. 1. Inverted SMM representation. The system is composed by a grounded AFM probe controlled by a laser-photodetector system. Sample is deposited on the hot electrode of a gold slot line. Sample holder is connected to a two port VNA through coaxial cables.](image)

The sample is positioned on the excited (hot) conductor of the high frequency line. The perturbation induced by the probe modifies the reflected and transmitted signals at the input and output of the line. This way transmitted and reflected signals can be recorded. From the equivalent circuit point of view, the tip-sample system is seen as a lossy capacitor shunting the slot line. Conventional SMMs usually work in reflection mode only and both excitation and measurements are performed directly through the AFM tip. Compared with conventional SMM, iSMM has some advantages including broader bandwidth, higher sensitivity, and wider dynamic range [18].

B. Materials and instruments

The sapphire substrate has dimensions of 2.4 cm × 2.4 cm × 0.03 cm. The gold lines are 1.2-μm thick and 80-μm wide, separated by a 10-μm gap. The lines are terminated in SMA connectors at the input and output, capacitively coupled. Coaxial cables are used to couple the connectors to an Agilent Technologies N5230A PNA-L VNA operating between 10 MHz and 20 GHz. The AFM probe is a Rocky Mountain Nanotechnology 12Pt400A full metal tip specially designed for SMM applications and electrically grounded for iSMM.

Jurkat cells were deposited onto the sapphire sample holder and cultured in a petri dish for more than 24 h under 37 °C and 5% CO2 in Advanced RPMI 1640. After that, cells were washed by phosphate-buffered saline solution to reduce salt deposits on the sample holder. Finally, the cells were dried in a sterile environment for more than 24 h.

III. Results

A. iSMM imaging

Fig. 2 shows AFM topography and simultaneously recorded raw iSMM S11 images at 4 GHz of a Jurkat cell. Results are shown in terms of amplitude, phase, real and imaginary part of reflection coefficient. The topography section crossing the center of the cell is shown in Fig. 2 (b), indicating the presence of sample between y = 2 μm and y = 16 μm. A good contrast is observed in S11 images between regions with and without sample at the selected frequency. Single line profiles for the acquired S11 are also shown in Fig. 2 (e,h). It can be seen (mostly from single line graphs) that the iSMM images reveal additional features beyond the sample topography. These characteristics are related to superficial and sub-surface variation of electrical properties. Despite the image quality is good, a certain number of sample details are likely missing in the high frequency data. This is due to the large size of the analyzed sample, and in particular it is expected iSMM to reduce resolution for thick samples. By shrinking the scanned area over a thinner part of the sample, a higher number of sample details would be visible. However, our aim is to characterize the cell entirely, thus a large scan area was preferred. To quantitatively interpret SMM data, raw images are calibrated; the obtained quantities are used to extract intrinsic sample parameters.

B. iSMM calibration

S11 images were calibrated following the algorithm described in [13]. The calibration procedure consists of measuring an S11 approach curve on bare gold without any sample, shown in Fig. 3 (a) in terms of amplitude and phase. The curve is then converted to a capacitance approach curve depicted in Fig. 3 (b). Note that in this case, the resulting calibrated admittance is purely capacitive because the tip and metal plane are separated by air only.
Fig. 2. (a) AFM topography of a Jurkat cell. Yellow regions indicate presence of cell, red-black areas correspond to the gold line. (b) Topography cross section corresponding to the blue dotted line shown in panel (a). Simultaneously acquired high frequency data in terms of (c) amplitude, (d) phase, (f) imaginary part and (g) real part based on $S_{11}$ at 4 GHz. Profiles are shown in Fig. (e) and (h) along the same cross section as (b).

Fig. 3. (a) Raw approach curve based on $S_{11}$ at 4 GHz. (b) Calibrated capacitance curve compared with analytical model of sphere on top of conductive plane. Calibrated $\text{Re}(Y)$ (c) and $\text{Im}(Y)/\omega$ (e) image of the Jurkat cell. Single line contrast for $\text{Re}(Y)$ and $\text{Im}(Y)/\omega$ are respectively shown in Fig. (d) and (f). Contrast is taken with respect to a point where no sample is present. Capacitance approach curve agrees with the analytical model of a sphere on top of a ground plane [20] confirming the accuracy of calibration. Fig. 3 (c,e) shows the calibrated admittance real and imaginary part (divided by angular frequency) of the Jurkat cell. Line contrast across the middle of Fig. 3 (c,e) is depicted in Fig. 3 (d,f) along the $y$ direction. Differences are taken with respect to a spatial point in which no sample is present. The $\text{Re}(Y)$ contrast from 2 μm to 16 μm is explained by the presence of the cell with non-zero conductive properties. In fact, conductivity of sample provokes the increase of $\text{Re}(Y)$ w.r.t. points where the sample is not present and in which $\text{Re}(Y)=0$. On the other hand, the negative $\text{Im}(Y)/\omega$ contrast on cell is due to the tip vertical movement, increasing the tip-metal substrate distance in the presence of sample (commonly called topography crosstalk).

The absolute values reported in Fig. 3 depends not only on the electrical properties of the sample, but also on the probe geometry, especially on the effective tip radius $r$. Note that this latter differs from the nominal geometrical radius being an effective parameter. In the present case, the effective radius selected for calibration is 620 nm. This value is obtained by measuring an Electrostatic Force Microscopy (EFM) approach curve on a conductive surface. The force curve is then converted in $dC/dz$ curve, in which $C$ indicates the tip-metal substrate capacitance and $z$ is the tip-ground separation. The radius is estimated by fitting the measured data with the analytical model of the capacitance between the point-ball tip and the conductive reference plane.
C. Sample local dielectric constant

Intrinsic sample electrical parameters are derived from calibrated \( \text{Re}(Y) \) and \( \text{Im}(Y)/\omega \). In particular, local dielectric constant is evaluated from calibrated \( \text{Im}(Y)/\omega \) by properly correcting the effect of topography. The topography cross-talk contribution \( C_{\text{cross}} \) is defined as the capacitance between the tip and the ground when their distance corresponds to the sample height. In other words, \( C_{\text{cross}} \) is the capacitance obtained when the tip scans only on air but with the same topography of the real sample. Following the definition of \([21]\), sample dielectric constant can be derived from difference between calibrated \( \text{Im}(Y)/\omega \) and topography cross-talk contribution:

\[
\varepsilon_R = \frac{\text{Im}(Y)/\omega - C_{\text{cross}}}{2\pi\epsilon_0 r}.
\] (1)

Fig. 4 shows the obtained \( \varepsilon_R \) map, indicating an almost homogeneous dielectric constant of around 2.6 ± 0.3 in the correspondence of the cell. The estimated nominal value is in general agreement with previous results obtained for dried L6 cells by means of conventional SMM technique \([22]\).

Fig. 4. Dielectric constant map of the dried Jurkat cell. The cell shows homogeneous dielectric properties indicating a dielectric constant of around 2.6 ± 0.3 nearby its centre.

IV. CONCLUSION

Microwave imaging and local complex admittance mapping of a single dried Jurkat cell is reported. The work has been developed by a recently published inverted SMM. Measured \( S_{11} \) data is showing good sensitivity and quality for imaging the cell. A calibration algorithm originally developed for conventional SMM has been applied and complex local admittance of the cell is obtained. Finally, dielectric constant of the sample is mapped and shown to be around 2.6 ± 0.3 and homogeneous along the cell surface.

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