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Inverted scanning microwave microscope for *in vitro* imaging and characterization of biological cells

Marco Farina,[1,a)] Xin Jin,[2] Gianluca Fabi,[1] Eleonora Pavoni,[1] Andrea di Donato,[1] Davide Mencarelli,[1] Antonio Morini,[1] Francesco Piacenza,[3] Richard Al Hadi,[4] Yan Zhao,[4] Yaqing Ning[2], Tiziana Pietrangelo,[5] Xuanhong Cheng,[6] and James C. M. Hwang[2]

[1]*Dept. of Information Engineering, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy*

[2]*Dept. of Electrical and Computer Engineering, Lehigh University, Bethlehem, PA 18015 USA*

[3]*Advanced Technology Center for Aging Research, Scientific Technological Area, IRCCS INRCA, Via Birarelli 8, 60121 Ancona, Italy*

[4]*Alcatera LLC, 1401 Westwood Blvd, Suite 280, CA 90024, USA*

[5]*Dept. of Neuroscience, Imaging and Clinical Sciences, University G d'Annunzio Chieti-Pescara, I-66013 Chieti, Italy*

[6]*Dept. of Materials Science and Engineering and Dept. of Bioengineering, Lehigh University, Bethlehem, PA 18015 USA*

This paper presents an instrument called an inverted scanning microwave microscope (iSMM), which is capable of noninvasive and label-free imaging and characterization of the intracellular structures of a live cell on the nanometer scale. In particular, the iSMM is sensitive to not only surface structures, but also electromagnetic properties up to one micrometer below the surface. Conveniently, the iSMM can be constructed through straightforward conversion of any scanning probe microscope, such as the atomic force microscope and the scanning tunneling microscope, with a simple metal probe to outperform a traditional SMM in terms of ruggedness, bandwidth, sensitivity and dynamic range. By contrast, the application of the traditional SMM to date has been limited to mainly surface physics and semiconductor technology, because the traditional SMM requires a fragile and expensive probe and is incompatible with saline solution or live cells.

[a)] E-mail: m.farina@univpm.it

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In a scanning probe microscope such as the atomic force microscope (AFM) and the scanning tunneling microscope (STM), a probe in the form of a sharp stylus is scanned across the sample within 1 nm of its surface, while variation of the interaction between the probe and the sample is recorded.[1] For example, variations of force and current are recorded in the AFM and the STM, respectively. The interaction can also be through an evanescent electromagnetic field, such as in the scanning near-field optical microscope (SNOM)[2] and the scanning microwave microscope (SMM).[3] In these cases, the spatial resolution is determined by the sharpness of the probe rather than the optical or microwave wavelength. Despite the much longer microwave wavelength than the optical wavelength, the SMM has the following advantages[4] over the SNOM: (i) noninvasiveness because the energy of microwave photons is on the order of 10 μeV, (ii) sensitivity to optically opaque materials, (iii) spectroscopy over many decades of frequencies using the same broadband microwave source, and (iv) sensitivity to dielectric permittivity of frequencies relevant to most electronic and biological functions. For these advantages, the SMM can be explored for many other applications beside the current focus on surface physics and semiconductor technology.[5]

In principle, the most impactful application of the SMM is in noninvasive, label-free imaging and characterization of live cells, organelles, bacteria and viruses. However, so far there have been only a few reports on the application of SMM in biology, and they are limited to dead or barely surviving samples.[6,7] This is mainly because the microwave probe is often incompatible with the saline solution necessary to keep a cell alive. Even if the probe survives the saline solution, it is rendered insensitive by the parasitic interaction between the probe body and the surround [Fig. 1(a)].[8,9] In fact, because a conducting sample holder is usually used to maximize the reflection of the microwave signal, the parasitic interaction between the probe body and the surround can be orders-of-magnitude larger than the intrinsic interaction between the probe tip and the sample. It is even worse when the probe is immersed in the saline solution, because the solution has a much higher dielectric permittivity than that of air. Currently, to boost the sensitivity of an SMM amidst the parasitic interaction, a resonance circuit is often used,[4] which precludes broadband spectroscopy. However, broadband SMM is desirable because (i) it provides information at relevant frequencies, (ii) it allows time-gated filtering of unwanted signals through post-measurement data processing,[6] and (iii) it enables microwave tomography.

FIG. 1. Schematics of (a) a traditional AFM-modified SMM and (b) an inverted SMM. In (a), one-port microwave measurement is performed through the AFM probe, which suffers from parasitic interaction between the probe body and the surrounding ground. The parasitic interaction is aggravated when the probe is immersed in saline solution. In (b), two-port microwave measurement is performed through the input and output ports of a coplanar waveguide (CPW) as part of the sample holder. The

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parasitic interaction between the probe body and the surround is minimized because the probe is grounded like the ground electrodes of the CPW.

Typically, an SMM is modified from an AFM or an STM. In either case, the microwave signal is injected through the probe by a microwave generator or a vector network analyzer (VNA), and the signal reflected from the sample is also sensed by the VNA. The ratio of the reflected and the injected signals (the reflection coefficient) can be used to determine the spreading resistance or dielectric permittivity of the sample, after proper calibration and analysis. Such a one-port reflection measurement usually has a dynamic range of 40–60 dB.

To minimize the parasitic interaction and to boost the SMM sensitivity without resorting to a resonance circuit, we propose a technique called an inverted SMM (iSMM). As shown schematically in Fig. 1(b), in an iSMM, the scanning probe is always grounded, and the microwave signal is injected through a transmission line such as a coplanar waveguide (CPW) or a slot line as part of the sample holder. (For high sensitivity, it is preferable to concentrate the sample on the center electrode of the CPW or the excited electrode of the slot line. Details of the experimental setup can be found in *Supplementary Material*.) Unlike the traditional SMM probe, the transmission line can have broadband impedance match over many decades of frequency.[10] The input and output of the transmission line are connected to the VNA, so that both reflection and transmission coefficients are measured. Such a two-port measurement usually has a dynamic range of 120–140 dB, which makes it easier to sense the tiny perturbance when the probe scans across the sample.

According to the reciprocity theory of electromagnetics, the intrinsic interaction between the probe tip and the sample is the same whether the microwave signal is injected through the probe or the sample. However, with the microwave signal injected through the sample and the probe grounded, the parasitic interaction between the probe body and the surround is greatly reduced, because most of the surround is grounded in any case. Thus, compared to a traditional SMM, an iSMM can have wider dynamic range, higher sensitivity, and broader bandwidth (by rendering a resonance circuit unnecessary). Additionally, the probe can be a simple, rugged and bio-compatible metal stylus. Meanwhile, whether the iSMM is modified from an AFM or STM, the original AFM or STM function is intact so that an iSMM image can be obtained simultaneously with an AFM or STM image.

To demonstrate the technique, we used an AFM-based iSMM on Jurkat human lymphocyte cells (dried only) and L6 rat myocyte cells (both live and dried). The detailed procedure is described in *Supplementary Material*. Fig. 2 compares the AFM and iSMM images of dried Jurkat cells. It can be seen that the quality of the iSMM image is at least as good as that of the AFM image.

FIG. 2. Simultaneous (a) AFM and (b) iSMM images of dried Jurkat cells. The iSMM image is based on the magnitude of the reflection coefficient at 4 GHz.

Fig. 3 compares the AFM and iSMM images of a live L6 cell in saline solution. The main difference between AFM and iSMM is that iSMM is sensitive to the properties of intracellular structures below the surface, so that, after proper calibration, the iSMM can quantify the intracellular permittivity. The iSMM image is also among the best-quality images formed by the transmission coefficient measured by a two-port SMM.[11,12] Detailed discussion on 2-port SMM can be found in *Supplementary Material*.

FIG. 3. Simultaneous (a) AFM and (b) iSMM images of a live L6 cell in saline solution. The iSMM image is based on the magnitude of the transmission coefficient at 3.4 GHz.

The calibration of SMM is not trivial and SMM has been used mostly for imaging instead of quantitative characterization. This is because most SMM analysis considers only the intrinsic interaction between the probe tip and the sample, ignoring the parasitic interaction between the probe body and the surround. Using an innovative calibration procedure[12] detailed in *Supplementary Material*, Fig. 4 illustrates the effect of calibration on the iSMM image of a dried L6 cell. Fig. 4(a) is the AFM topography image; Fig. 4(b) is the iSMM capacitance image corrupted by the sample topography; Fig. 4(c) is the iSMM dielectric constant image with the topography effect removed. As expected, the dried cell exhibited ridges near its periphery, but rather uniform dielectric constant of 2.8 ± 0.7 across the cell. This value is comparable to that of lipid bilayers in electrolyte solution,[14] but is lower than that of dried E. coli bacteria.[15]

FIG 4. (a) AFM topography, (b) iSMM capacitance, and (c) iSMM dielectric constant images of a dried L6 cell. The iSMM image is based on the magnitude of the transmission coefficient at 6.2 GHz.

In conclusion, we have experimentally demonstrated the iSMM for imaging and quantitative characterization on the nanometer scale, which can be applied through a straightforward modification of any scanning probe microscope. The technique is label-free, non-invasive, broadband, and highly sensitive, especially to subsurface electromagnetic properties. With a simple and rugged metal probe that is always grounded, it can be easily made biocompatible. Using the iSMM, a live cell in saline solution was imaged which has not been possible with a traditional SMM. Thus, the iSMM should broaden the application of SMM to many applications beyond the current focus on surface physics and semiconductor technology. With the broadband capacity of iSMM, work is in

progress for microwave tomography, the equivalent of optical coherent tomography but with far less invasive microwave photons.

SUPPLEMENTARY MATERIAL

See supplementary material for details of experimental setup, cell preparation, data processing, and comparisons between S_{11} and S_{21} images.

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REFERENCES

[1]G. Binnig, C. F. Quate, and C. Gerber, *Phys. Rev. Lett.* **56**, 930 (1986).
[2]A. L. Lereu, *Int. J. Nanotechnol.* **9**, 488 (2012).
[3]E. A. Ash and G. Nicholls, *Nature* **237**, 510 (1972).
[4]G. Gramse, E. Brinciotti, A. Lucibello, S. B. Patil, M. Kasper, C. Rankl, R. Giridharagopal, P. Hinterdorfer, R. Marcelli, and F. Kienberger, *Nanotechnol.* **26**, 135701 (2015).
[5]E. Brinciotti, G. Gramse, S. Hommel, T. Schweinboeck, A. Altes, M. A. Fenner, J. Smoliner, M. Kasper, G. Badino, S.-S. Tuca, and F. Kienberger, *Nanoscale* **7**, 14715 (2015).
[6]M. Farina, A. Lucesoli, T. Pietrangelo, A. di Donato, S. Fabiani, G. Venanzoni, D. Mencarelli, T. Rozzi, and A. Morini, *Nanoscale* **3**, 3589 (2011).
[7]S.-S. Tuca, G. Badino, G. Gramse, E. Brinciotti, M. Kasper, Y. J. Oh, R. Zhu, C. Rankl, P. Hinterdorfer, and F. Kienberger, *Nanotechnol.* **27**, 135702 (2016).
[8]S. M. Anlage, V. V. Talanov, and A. R. Schwartz, in *Scanning Probe Microscopy*, edited by S. Kalinin and A. Gruverman (Springer, New York, NY, USA, 2007), p. 215.
[9]K. Lai, W. Kundhikanjana, M. Kelly, and Z. X. Shen, *Rev. Sci. Instrum.* **79**, 063703 (2008).
[10]X. Ma, X. Du, H. Li, X. Cheng, and J. C. M. Hwang, *IEEE Trans. Microw. Theory Techn.* **66**, 3690 (2018).
[11]A. O. Oladipo, A. Lucibello, M. Kasper, S. Lavdas, G. M. Sardi, E. Proietti, F. Kienberger, R. Marcelli, and N. C. Panoiu, *Appl. Phys. Lett.* **105**, 133112 (2014).
[12]L. Zheng, D. Wu, X. Wu, and K. Lai, *Phys. Rev. Appl.* **9**, 061002 (2018).
[13]M. Farina, D. Mencarelli, A. Di Donato, G. Venanzoni, and A. Morini, *IEEE Trans. Microw. Theory Tech.* **59**, 2769 (2011).
[14]G. Gramse, A. Dols-Perez, M. A. Edwards, L. Fumagalli, and G. Gomila, *Biophys. J.* **104**, 1257 (2013).
[15]M. C. Biagi, R. Fabregas, G. Gramse, M. Van Der Hofstadt, A. Juárez, F. Kienberger, L. Fumagalli, and G. Gomila, *ACS Nano* **10**, 280 (2016).
[16]M. C. Biagi, G. Badino, R. Fabregas, G. Gramse, L. Fumagalli, and G. Gomila, *Phys. Chem. Chem. Phys.* **19**, 3884 (2017).
[17]W. R. Smythe, *Static and Dynamic Electricity*, (McGraw-Hill, New York, NY, USA, 1950).