Integrated Fluorescence and Scanning Microwave Microscopy: Nano-Imaging with "Proof of Life"

Zahra Nemati‡1, Jinfeng Li†2 Peter J. Burke¶*3

‡Department of Chemical Engineering and Materials Science, † Department of Physics and Astronomy, ¶Integrated Nanosystems Research Facility, * Electrical Engineering and Computer Science, University of California, Irvine, Irvine, CA 92697, USA

pburke@uci.edu

***Abstract* — We demonstrate imaging using scanning microwave microscopy (SMM) of vital single cells in salt buffer. HeLa cells are cultured on 130 μm glass cover slips and left overnight in the incubator for optimal attachment. We verify that the cells are "alive" by measuring the membrane potential of mitochondrial using a voltage sensitive fluorescent dye (TMRE). The cells are measured capacitively at 7 GHz. We further track SMM map changes by adding digitonin-a detergent solution- to cells that would empty out cell content by creating holes on the plasma membrane. This paper represents a dramatic improvement of the technique of scanning microwave microscopy by integrating it with simultaneous fluorescence images of living cells to obtain "proof of life" via fluorescence imaging of HeLa cells which are simultaneously imaged via SMM. This integration and combination of the two techniques (fluorescence microscopy and scanning microwave microscopy) should enable the entire fluorescence bioimaging technology to be combined with SMM for simultaneous optical and SMM images of biological processes.**

***Keywords* — fluorescence microscopy, microwave in liquid, cancer biology, live cell imaging.**

I. Introduction

The Scanning microwave microscopy[1] has the potential to non-invasively peer into living cells. It has already been demonstrated to penetrate into materials at different depths based on frequency[2]. However, a challenge has been to integrate the AFM portion with traditional optical fluorescence microscopy and hence provide "proof of life" that the SMM is imaging something alive.

Prior work on imaging cells in liquid by Keysight showed calibrated capacitance measurements of E-Coli and CHO cells in PBS[3]. (The signal processing that is used to deduce practical information from images such as calibrated capacitance density is based on a calibration standard that is described in [3].) That work was done on a silicon non-transparent substrate. Additional work by Farina and colleagues demonstrated [4],[5][6][7][8] imaging of breast cancer and muscle cells, as well as exosomes. Imaging of vital mitochondria was presented by our lab in 2018 [9]. All of these have demonstrated the potential of SMM to image living systems.

In this paper, we dramatically improve the technique by integrating it with simultaneous fluorescence images of living cells to obtain "proof of life" via fluorescence imaging of HeLa cells which are simultaneously imaged via SMM.

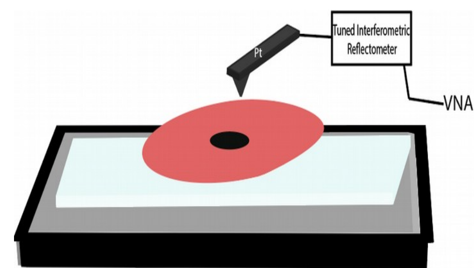

Figure 1: Cartoon of a single HeLa cell attached to the surface of a 130um thick glass coverslip with AFM tip from the top and fluorescent objective in the bottom. The red indicates the fluorescence signal of TMRE dye.

II. Materials and Methods

A. HeLa Cell Culture and Preparation

HeLa cells of low passage numbers were cultured in standard DMEM media in 37°C incubator, tagged with TMRE and grown on glass cover slips for dual imaging. The average HeLa cell is less than 20 μm in diameter, which would make the atomic force microscopy and hence SMM mapping of a single cell possible.

B. Fluorescent Imaging

Fluorescent imaging was done via our Olympus fluorescent microscope, which is incorporated into the AFM system to allow for a simultaneous imaging platform. In order to monitor the vitality of cells and change in their morphology, HeLa cells were tagged with red/orange TMRE potentiometric dye with excitation/emission maximum of 549/574 nm.

III. Scanning Microwave Microscope

The scanning microwave microscope, from Keysight™ (model 7500) consists of an AFM interfaced with a performance vector network analyser, as shown in Fig. 1-3.
A microwave signal is transmitted from the PNA to a conductive AFM probe that operates in tapping mode with the sample being scanned. The probe also serves as a receiver to capture the reflected microwave signal from the contact point. By directly measuring the complex reflection coefficient, the impedance of the sample at each scanned point can then be mapped, simultaneously with the surface topography[1]. In the proposed configuration, a homemade tuned interferometric system is developed to control the interference frequency position and the level of the magnitude of the reflection

Authorized licensed use limited to: Access paid by The UC Irvine Libraries. Downloaded on June 06,2020 at 19:20:01 UTC from IEEE Xplore. Restrictions apply.

coefficient. The interferometer is built up in coaxial form with a hybrid coupler, passive and variable phase-shifter and attenuator, and a low-noise amplifier. Initial images have been obtained on isolated mitochondria [9].

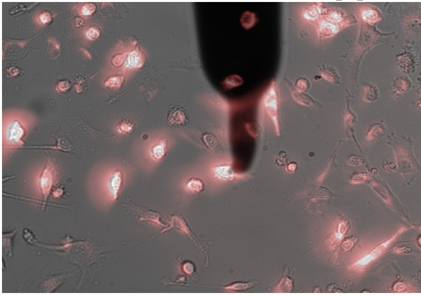

Figure 2: Superimposed image of the AFM/SMM tip on top of a single vital HeLa cell

IV. SINGLE CELL STUDIES

C. Verification of Cell Vitality Using Fluorescent Imaging

Fig. 2 shows single HeLa cancer cells fluorescently tagged with TMRE potentiometric dye. The cells were then incubated overnight to allow for proper attachment to the glass surface to facilitate AFM scanning. The TMRE potentiometric dye is a cell permeant, positively charged dye that accumulates in active mitochondria due to their negative charge and fails to do so in inactive or depolarized mitochondria given the diminished charge. As a result, a TMRE fluorescence signal is indicative of the vitality of mitochondria in the cell and thereby the cell.

D. SMM Characterization of Single Cells

The device was brought into contact with the SMM probe in tapping mode with gentle tip-sample interaction to prevent displacement or damage of single cells. Previously we did a tuning of the reflectometer and selected the inference peak at ~7 GHz for mitochondrial imaging and acquired an uncalibrated capacitance map of a single vital mitochondria[9] that will be applied to these cells.

V. DISCUSSION

A. Comparison to prior art

Several previous works (https://arxiv.org/pdf/1802.05939.pdf;www.ncbi.nlm.nih.gov/.../articles/PMC6469850;www.ncbi.nlm.nih.gov/.../articles/PMC5424529), including our own[9], have demonstrated SMM in liquid. Our work even demonstrated it with isolated mitochondria. However, what is new about this work is that we can image the cell/mitochondria simultaneously with the optical fluorescence image, hence providing "proof of life". Our prior work imaged them separately (i.e. optical in one system, and SMM in another system).

B. Benefits of dual imaging system

The ability to image with fluorescence enables all of the technology developed in molecular biology to tag various cell properties. This enables cross-checking of the new SMM technology with existing proven techniques in biological imaging.

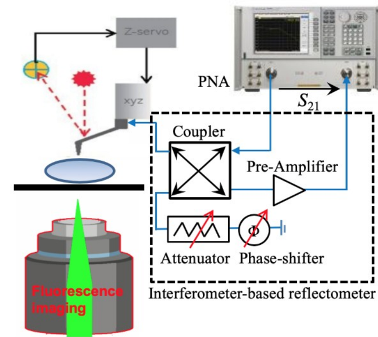

Figure 3: Diagram of the SMM setup with the inverted fluorescence microscope in conjunction.

ACKNOWLEDGMENTS

The authors acknowledge support from the ARO W911NF-11-1-0024, W911NF18-1-0076, and W911NF-15-1-0376), NIH (CA182384), and the French American Cultural Exchange (FACE) Partner University Fund program.

REFERENCES

[1] S. Wu, F. Kienberger, and H. Tanbakuchi, "Scanning Microwave Microscopy: Advances In Quantitative Capacitance And Carrier Density Measurements At The Nanometer Scale," in *Scanning Probe Microscopy for Energy Research*, D. A. Bonnell and S. V. Kalinin, Eds. Singapore: World Scientific, 2013, pp. 481–512.

[2] C. Plassard *et al.*, "Detection of defects buried in metallic samples by scanning microwave microscopy," *Phys. Rev. B*, vol. 83, no. 12, p. 121409, Mar. 2011.

[3] S.-S. Tuca *et al.*, "Calibrated complex impedance of CHO cells and E . coli bacteria at GHz frequencies using scanning microwave microscopy," *Nanotechnology*, vol. 27, no. 13, p. 135702, Apr. 2016.

[4] X. Jin *et al.*, "Imaging of exosomes by broadband scanning microwave microscopy," in *2016 46th European Microwave Conference (EuMC)*, 2016, pp. 1211–1214.

[5] M. Farina *et al.*, "Investigation of Fullerene Exposure of Breast Cancer Cells by Time-Gated Scanning Microwave Microscopy," *IEEE Trans. Microw. Theory Tech.*, vol. 64, no. 12, pp. 4823–4831, Dec. 2016.

[6] M. Farina *et al.*, "Tomographic effects of near-field microwave microscopy in the investigation of muscle cells interacting with multi-walled carbon nanotubes," *Appl. Phys. Lett.*, vol. 101, no. 20, p. 203101, Nov. 2012.

[7] M. Farina, A. Di Donato, D. Mencarelli, G. Venanzoni, and A. Morini, "High Resolution Scanning Microwave Microscopy for Applications in Liquid Environment," *IEEE Microw. Wirel. Components Lett.*, vol. 22, no. 11, pp. 595–597, Nov. 2012.

[8] M. Farina, D. Mencarelli, A. Di Donato, G. Venanzoni, and A. Morini, "Calibration Protocol for Broadband Near-Field Microwave Microscopy," *Microw. Theory Tech. IEEE Trans.*, no. 99, p. 1, 2010.

[9] J. Li, Z. Nemati, K. Haddadi, D. C. Wallace, and P. J. Burke, "Scanning Microwave Microscopy of Vital Mitochondria in Respiration Buffer," in *IEEE/MTT-S International Microwave Symposium*, 2018, pp. 115–118.

[10] T. Dargent *et al.*, "An interferometric scanning microwave microscope and calibration method for sub-fF microwave measurements," *Rev. Sci. Instrum.*, vol. 84, no. 12, p. 123705, Dec. 2013.

Authorized licensed use limited to: Access paid by The UC Irvine Libraries. Downloaded on June 06,2020 at 19:20:01 UTC from IEEE Xplore. Restrictions apply.