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# Inverted Scanning Microwave Microscopy of a Vital Mitochondrion in Liquid

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**Abstract**— The inverted scanning microwave microscope (iSMM) is a recently developed variety of scanning microprobe microscopes. Similar to an SMM, the iSMM is sensitive to not only surface structure, but also electromagnetic properties below the surface. Different from the SMM, the iSMM can be converted from any scanning probe microscope, such as an atomic force microscope (AFM) and a scanning tunneling microscope, with a simple metal probe to outperform the SMM in terms of ruggedness, bandwidth, sensitivity, and dynamic range. This paper reports the first iSMM images of a vital mitochondrion in an isotonic glucose solution. Compared to the AFM and SMM, the iSMM offers more insights into the mitochondrion. This shows the potential of iSMM for noninvasive and label-free imaging and characterization of subcellular structures.

**Index Terms**— Atomic force microscopy, biological materials, microwave imaging, mitochondria, scanning microwave microscopy.

## I. INTRODUCTION

THE SCANNING microwave microscope (SMM) [1], is a variety of scanning probe microscopes [2] such as the atomic force microscope (AFM) and the scanning tunneling microscope (STM). Unlike the AFM and the STM, the SMM is based on the near-field electromagnetic interaction between a nanoprobe and a sample. Typically, a vector network analyzer (VNA) is used to radiate a microwave signal through the probe, and, after near-field interaction with the sample, the reflected signal is sensed by the same probe and VNA.

By contrast, in the recently developed inverted SMM (iSMM) [3], [4], the probe is grounded and is used to perturb the signal propagating along a transmission line as the probe scans the sample above the transmission. Based on the measured changes in the reflection and transmission coefficients,  $S_{11}$  and  $S_{21}$ , the electromagnetic properties of the sample can be extracted. Compared to the SMM, the iSMM can

be more rugged, more sensitive, and with wider bandwidth and dynamic range.

The SMM has been used to characterize live biological cells, despite the challenges for operating the SMM in a physiological buffer necessary to keep the cells alive [5], [6]. It has also been used to characterize subcellular organelles such as a mitochondrion [7], which is found in large numbers in most cells. By characterizing the structure and properties of a mitochondrion undergoing the biochemical processes of respiration and energy production, we can better understand the metabolism of a cell and, ultimately, a human being [8]. In [7], the SMM image shows that the outer morphology of a mitochondrion is consistent with that measured by an AFM, but does not contain additional insight into the mitochondrion despite the potential advantage of the SMM for below-surface sensitivity. In this work, we use the iSMM to better overcome the challenges for in-liquid operation, and image for the first time a vital mitochondrion with more details than that by using an SMM or AFM. The mitochondrion is fully immersed in liquid to ensure its vitality while undergoing AFM and iSMM for hours. The AFM and iSMM are operated in a non-contact mode to avoid deforming the mitochondrion.

This work focuses on demonstrating the iSMM technique in liquid and in a non-contact mode. Except in [5], most SMM biological studies are performed with the sample dried or enveloped in a drop of liquid [6], so that scanning must be completed in minutes making it impossible to calibrate the data for quantitative analysis or to observe a vital sample over time as it undergoes physiological change. Moreover, typical AFM and SMM are performed in the contact mode, which can achieve higher sensitivity but can deform soft samples such as a living cell. Here we use the non-contact mode without deforming the mitochondrion, but with sufficient sensitivity to reveal interior details. With the technique demonstrated, more experimental outcome can be expected in the future.

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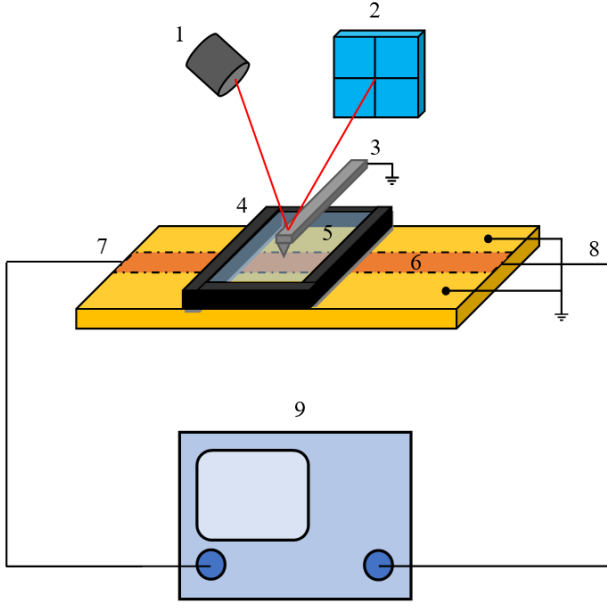
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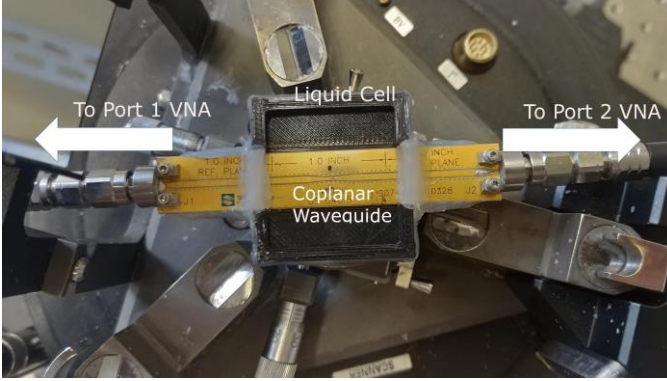
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(a)



(b)

Fig. 1. (a) Schematics and (b) photograph of an AFM-based iSMM with a liquid cell on a CPW transmission line. 1. Laser. 2. Photodetector. 3. AFM probe. 4. Liquid cell. 5. Physiological buffer. 6. CPW center electrode. 7. To VNA port 1. 8. To VNA port 2. 9. VNA.

## II. EXPERIMENTAL SETUP AND SAMPLE PREPARATION

### A. iSMM Setup

Fig. 1 shows that the iSMM comprises an NT-MDT Solver Pro-47 AFM and a coplanar waveguide (CPW). The CPW input and output are connected via SMA connectors and coaxial cables to the two ports of a Keysight Technologies E8361A 67-GHz PNA. Mitochondria in an isotonic glucose solution are deposited on the CPW center electrode and surrounded by a liquid cell. The signal-to-ratio ( $SNR$ ) of  $S_{11}$  is defined as

$$SNR(S_{11}) = 20 \log \left[ \frac{|S_{11}(z = 1 \text{ nm}) - S_{11}(z = 3 \text{ } \mu\text{m})|}{\sigma_{[S_{11}(z = 1 \text{ nm})]}} \right] \text{ dB} \quad (1)$$

where  $z$  is the height of the probe above the sample and  $S_{11}(z = 1 \text{ nm})$  and  $S_{11}(z = 3 \text{ } \mu\text{m})$  are measured with the probe near the

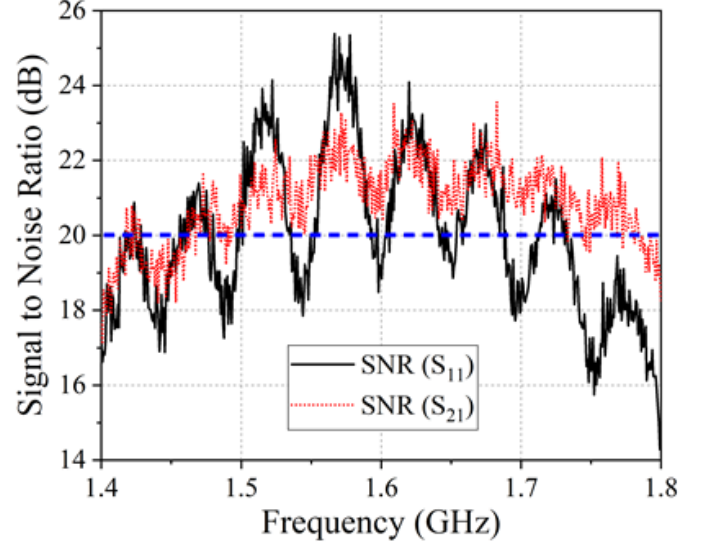


Fig. 2. Signal-to-noise ratios ( $SNR$ s) of  $S_{11}$  and  $S_{21}$  measured by the iSMM around 1.6 GHz.

signal line and 3  $\mu\text{m}$  above, respectively. Each measurement is repeated 500 times to evaluate the standard deviation  $\sigma$ . With  $S_{11}$  in the vector form in (1), the averaging accounts for noises in both amplitude and phase.  $SNR(S_{21})$  is similarly defined.

Experimentally, the best  $SNR$  is achieved around 6 GHz in air, but 1.6 GHz in liquid. Fig. 2 shows that the measured  $SNR(S_{11})$  and  $SNR(S_{21})$  between 1.5 GHz and 1.8 GHz are mostly above 20 dB, sufficient for high-quality images. The iSMM is performed in the non-contact mode to avoid deforming the mitochondria while allowing repeated measurements for the maximum  $SNR$ . Typically, it takes 10 min for the iSMM to scan an area of  $8 \times 8 \text{ } \mu\text{m}^2$  in  $256 \times 256$  pixels. The liquid cell has a capacity of several milliliters to last for hours without drying out.

### B. Sample Preparation

Mitochondria are isolated from a badge of one-million myogenic cells which are pelleted in 600-ml isolation buffer with 250-mM Saccharose, 20-mM Hepes, 1-mM EDTA, and 0.5% Bovine serum Albumin. The cells are then homogenized for 5 s using Turax and additional isolation buffer. Next, the cell suspension is centrifuged at 4000 rpm and 4  $^\circ\text{C}$  for 5 min. The supernatant containing mitochondria and vesicles is collected and the pellet containing cellular debris and nuclear material is discarded. Thereafter, the supernatant is centrifuged at 14000 rpm and 4  $^\circ\text{C}$  for 10 min. Discarding the supernatant, the pellet is resuspended in 500-ml isolation buffer with 300 ml of 0.01% Digitonin added. The resuspension is centrifuged at 14000 rpm and 4  $^\circ\text{C}$  for 10 min. The pellet is washed in 500 ml isolation buffer and centrifuged at 14000 rpm and 4  $^\circ\text{C}$  for 15 min. Finally, the pellet containing mitochondria is resuspended in an isotonic glucose solution to maintain their vitality.

For iSMM scan, mitochondria are deposited on the CPW with the aid of polylysine. First, a drop of polylysine is deposited on the CPW for 30 min before its residue is removed. Second,

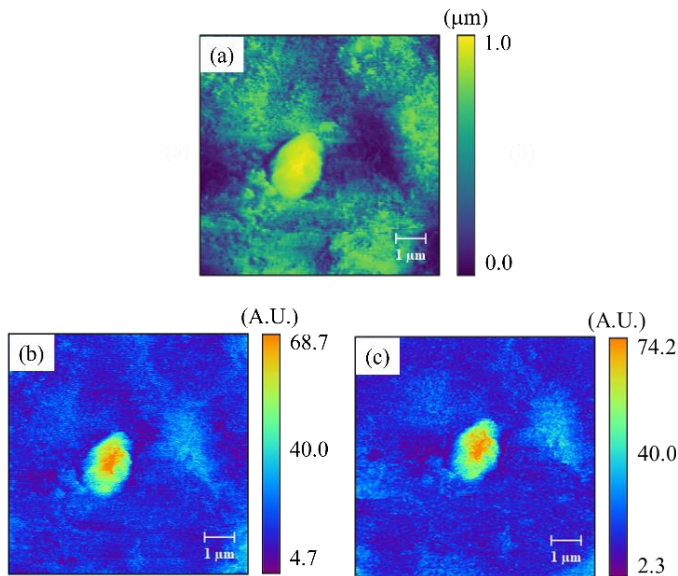


Fig. 3. (a) AFM topography, (b) iSMM  $|S_{11}|$ , and (c) iSMM  $|S_{21}|$  images of the same mitochondrion in an glucose isotonic solution.

10  $\mu\text{l}$  of glucose suspension of mitochondria are deposited on the polylysine and left to settle for 30 min before iSMM commences.

### III. RESULTS AND DISCUSSION

Fig. 3 compares the AFM topography image with the iSMM  $S_{11}$  and  $S_{21}$  images obtained simultaneously on the same mitochondrion approximately 1  $\mu\text{m}$  in diameter. The iSMM signals have been filtered and averaged over the frequency band of 1.6–1.8 GHz. It can be seen that the  $|S_{11}|$  and  $|S_{21}|$  images are of comparable quality, and both reveal details that are absent in the AFM image. Additionally, because the mitochondrion is not conductive, it is in high contrast against the surrounding CPW electrode.

Unlike most SMMs, the iSMM is capable of broadband measurements. This allows the  $S_{11}$  and  $S_{21}$  signals measured from 1.6 GHz to 1.8 GHz by the iSMM be transformed to the time domain by inverse Fourier transformation. Thereafter the unwanted signals can be gated out to further improve the SNR [9], [10]. Fig. 4 shows that the time-gated iSMM  $S_{11}$  and  $S_{21}$  images reveal finer details beyond that of Fig. 3.

From the measured  $S_{11}$  or  $S_{21}$ , the interaction impedance between the iSMM probe and the mitochondrion can be extracted. In turn, the local variation of the dielectric properties of the mitochondrion can be revealed as has been done for a live cell by the SMM [5]. However, the calibration procedure for quantitative iSMM in liquid has not been developed yet.

### IV. CONCLUSION

This paper presents the first time iSMM  $S_{11}$  and  $S_{21}$  images of a vital mitochondrion in an isotonic glucose solution. These images reveal details inside the mitochondrion not seen in the AFM topography image. Time-gated postprocessing of the iSMM  $S_{11}$  and  $S_{21}$  signals reveal even more details of the

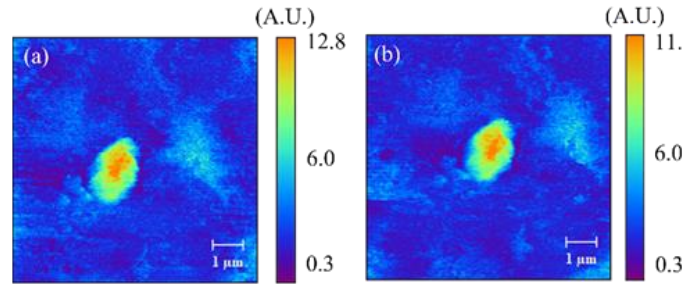


Fig. 4. Time-gated iSMM (a)  $|S_{11}|$  and (b)  $|S_{21}|$  images of the same mitochondrion as in Fig. 3.

mitochondrion. The measured  $S_{11}$  and  $S_{21}$  signals can be further processed to de-embed the dielectric properties of the mitochondrion in three dimensions and submicron scale. Similar approaches can be used to investigate in real time other subcellular structures such as the nucleus, chloroplasts, glycocalyx, and endoplasmic reticulum as a live cell undergoes physiological changes.

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