Martian Soil Biosensors Based on Dielectric Spectroscopy

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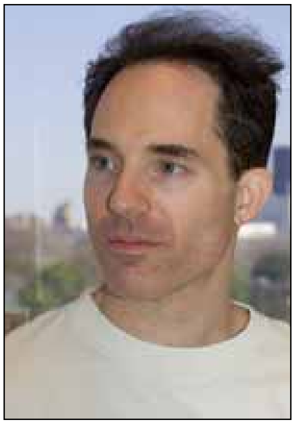
David Warmflash, Dharmakeerthi Nawarathna

Abstract—The goal is to investigate novel biosensing techniques, including dielectric spectroscopy and nonlinear harmonic response, which could ultimately be employed to develop instruments capable of detecting live organisms in samples from the outer terrestrial bodies of the solar system. Our previous results suggested that variable temperature dielectric spectroscopy can distinguish live organisms from nonliving complex macromolecules and may be suitable for *in situ* astrobiology studies on the surface of Mars or, eventually, in the liquid ocean beneath the ice of Europa. More recently, we have measured the frequency- and amplitude-dependent nonlinear harmonic responses of live cells, mitochondria, and chloroplasts, coupled with activator and inhibitor studies. Results provide compelling evidence that physiologically relevant processes in active macromolecular enzyme complexes are responsible for observed induced harmonics, thus providing additional signatures of live organisms.


Dr. John H. Miller, Jr.

The possible existence of life on Mars in the distant past or at present[1] has been of interest for well over a century. This issue has important scientific implications for the evolution of life on earth and the distribution of life in the cosmos. The Viking program, in 1976, made the first attempt to detect evidence of living or fossilized organisms in Martian soil and yielded ambiguous, somewhat negative results.[2] More recent studies[3] of the Martian meteorite Allan Hills 84001 (ALH84001) suggest that microbial life existed on Mars about four billion years ago. Compelling evidence includes the presence of magnetite crystals (Fe_3O_4) found in carbonate globules and their associated rims in the meteorite.[4] About one fourth of these tens-of-nanometer sized magnetites are nearly identical to those produced by magnetotactic bacteria on Earth and are not expected to be produced by abiotic means. It has, therefore, been argued that these Martian magnetite crystals are in fact magnetofossils, which, if true, would constitute evidence of the oldest life forms known.[5]

Additional findings suggest that subsurface Martian life could potentially survive even today.[6] There is abundant geological evidence that ice was once deposited in the regolith, where it should still be present above mid-latitudes.[7] This ice, which probably extends several kilometers below the surface, could be a source of liquid water near magmatic intrusions.[8] On Earth, the biomass of subterranean organisms may equal or exceed that at the surface.[9] These organisms can live in highly saline conditions at temperatures from 115°C to –20°C.[10,11] Such conditions might prevail beneath the surface in an aquifer or in a hydrothermal system. For these and other reasons, there is considerable interest in developing new techniques of detecting subsurface life on Mars. Moreover, the likelihood that oceans of liquid water exist below the icy surfaces of Europa and other moons makes these exciting candidates for the existence of extraterrestrial life in our solar system.


Dr. David Warmflash

Goal of the Project

The goal of this project is to investigate dielectric spectroscopy[12,13] and related methods, especially nonlinear harmonic response,[14] for the detection of live organisms. Toward this end, the project aims to elucidate possible signatures of active macromolecular complexes unique to living biological systems. Potential signatures include unusual behavior, distinct from those of inanimate materials, in the frequency- and temperature-dependent dielectric response, and in the induced nonlinear harmonic response as a function of frequency and amplitude.

Methodology

The previous report discussed the results of variable tempera-

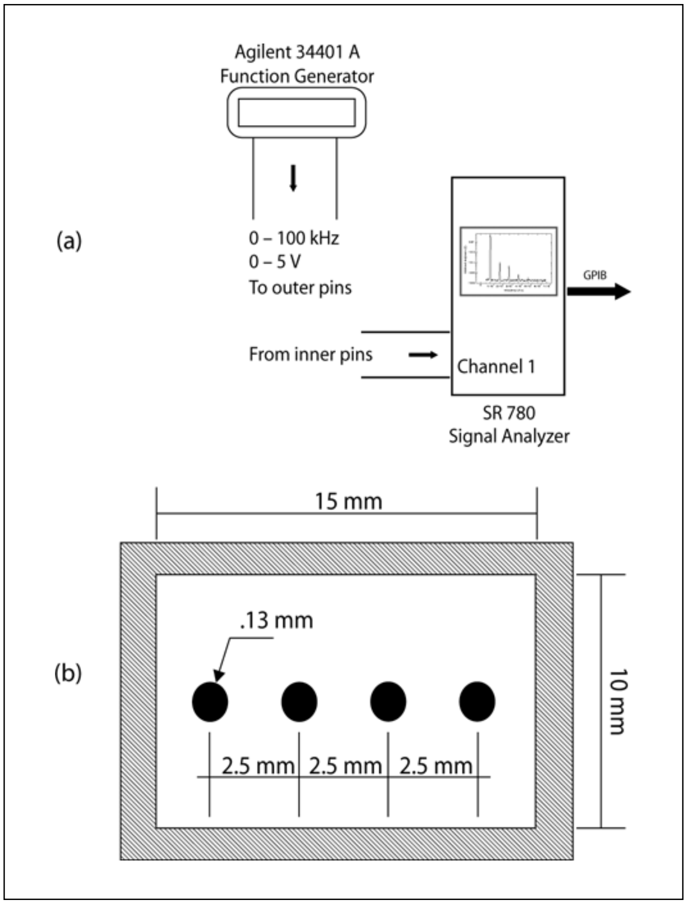


Figure 1. Four-electrode setup used to measure nonlinear harmonic response. (a) Experimental setup with external function generator and SR780 signal analyzer. (b) Planar, top view of the four-electrode sample cell showing the dimensions and geometry. The electrodes are 12-carat gold pins, 7 mm in length (cross-sections shown in figure).

ture dielectric spectroscopy of live organisms and Martian soil simulants. Our experiments on linear dielectric response employed a Solartron Impedance Analyzer, which measures complex admittance at frequencies up to 32 MHz. In this report, we focus on a study of potential signatures in the nonlinear harmonic response, which include signals produced by active molecular motors unique to live organisms.

For measurements at kilohertz frequencies, we employ a four-electrode system, utilizing a Stanford Research SR780 signal analyzer that we operate as a spectrum analyzer. A function generator applies a sinusoidal signal to the outer electrodes, while the voltage difference between the inner electrodes is fed into Channel 1 of the SR780, which records the induced harmonics. A reference spectrum is acquired using a supernatant, whose conductivity has been adjusted (with distilled water, to compensate for the volume fraction of the cells present in the sample) to be identical to that of the sample at the frequency of the interest. The supernatant typically consisted of an aqueous solution of ~1- 10 mM *NaCl*. Two different types of control files are used, depending upon whether the reference is to be logged using the same set of electrodes or a separate matched reference cell. In either case, the logging, windowing, and Fourier Transform routines were identical, and provide a power spectrum of the reference cell, which is also recorded as a data file in the computer. Finally, the sample power spectrum obtained from the sample (e.g., cell suspension or soil sample) of interest is divided by the reference power spectrum and also stored. The entire procedure is automated using LabVIEW data acquisition software. The power of this approach lay in allowing one to deconvolve the effects of nonlinearities within the electrochemical system from those due to the biological cells themselves.

Equipment and Special Technology

A diagram of our four-electrode setup for nonlinear response, including the Stanford Research SR780, is shown in Fig. 1. This setup enables us to obtain the frequency- and amplitude-dependence of harmonics induced by the sample (e.g., cell suspension) under investigation. At low frequencies, we employ a superconducting quantum interference device (SQUID) to directly probe the magnetic fields produced by the conduction and displacement currents. This reduces spurious harmonics generated at the electrode-medium interface, which become especially problematic at frequencies below 100 Hz. Details of this work have been reported in our 2004 study in *Applied Physics Letters*.[14]

Results and Discussion

We measured the nonlinear harmonic response of suspensions of budding yeast cells (*S. cerevisiae*), mitochondria (extracted from bovine heart and mouse liver cells), *B. indicas* (a prokaryotic relative of the mitochondrial ancestor), and chloroplasts (extracted from spinach) using the four-electrode setup shown in Fig. 1, in which a sinusoidal voltage is applied across the two outer electrodes, while the spectrum of induced harmonics is recorded across the two inner electrodes using a spectrum analyzer. When a suspension of cells or extracted organelles is excited using a single-frequency sinusoidal voltage excitation, a series of harmonics is produced for sufficient ac field amplitudes. Any background harmonics due to the electrode interface are subtracted out by measuring a reference medium with conductivity matched to that of the cell suspension.

When measuring the magnitudes of the induced second and third harmonic amplitudes vs. applied frequency for whole budding yeast cells, we observed two peaks that appeared to grow out of the background as cell concentration is increased. In addition, we found that rotenone, an inhibitor that affects complex I of the mitochondrial respiratory chain, reduces the amplitudes of the harmonics. We observed even more dramatic suppressing effects when adding potassium cyanide, a respiratory inhibitor that binds to the cytochrome *c* oxidase complex and essentially shuts down the entire electron transport chain on the mitochondrial inner membrane. Similar behavior is observed for *B. indicas*, a relative of the mitochondrial ancestor. Respiratory inhibitors suppress the mitochondrial transmembrane potential, hence shutting off the ATP-synthase molecular turbine, and prevents the production of ATP. Importantly, however, yeast cells are not killed by cyanide and remain capable of fermentation. In order to further study whether bioenergetic processes play a major role, we have performed similar measurements on isolated mitochondria extracted from mammalian cells.

Figure 2 shows the second harmonic response vs. applied frequency for uncoupled mammalian mitochondria, in which complex II has been activated through the addition of glutamate malate, whereas the ATP synthase complex is inactive due to the lack of a transmembrane potential and proton gradient. These results suggest that the peak in Fig. 2 may be attributed to electron/proton transport processes occurring between complex II and complex IV of the electron transport machinery. Note that the inhibitor rotenone quenches the harmonic response, as shown in Fig. 2. We also observe a higher frequency peak, appearing at applied frequencies of around 12 kHz in budding yeast cells and *B. indicas*, a relative of the mitochondrial ancester. In both of these cases, the ATP synthase complex is active, suggesting that the high frequency peak in these organisms may result from coupling to the remarkable molecular turbine in the F_0 unit of ATP synthase.

Photosynthetic organisms, including cyanobacteria and chloroplasts (responsible for photosynthesis in plants), are potentially exciting organisms to study because they utilize light rather than chemical energy to establish electrochemical gradients, allowing for greater flexibility and precision in controlling experimental conditions. Figure 3 cites results obtained for chloroplasts, showing dramatic changes in harmonic response with the presence or absence of light. These results strongly indicate that the observed behavior is caused by, in this case, active processes due to photosynthesis, and provide increased confidence of the physiological relevance of harmonic response measurements. Note, however, that the frequency-dependence is rather different from that observed for yeast cells and mitochondria, and may incorporate responses from the light harvesting complexes, reaction centers, and photosystems.

Conclusions

Our latest results suggest that ac electric fields, which capacitively couple through the plasma membrane, can interrogate bioenergetic and other processes in internal cellular organelles and potentially identify signatures of life. Additional experiments are planned to study coupled mitochondria, which should enable further confirmation of any signals arising from biological energy-transducing complexes. The observed unusual behavior, which in the case of chloroplasts is activated by light, provides signatures unique to live organisms and could ultimately be applied to *in situ* astrobiology studies on the surface of Mars or, eventually, in the liquid ocean beneath the ice of Europa. Additional terrestrial applications include fundamental research in biophysics, studies of the effects of drugs on live cells to aid in pharmaceutical development, studies of cancer cells, and the development of medical diagnostic instrumentation.

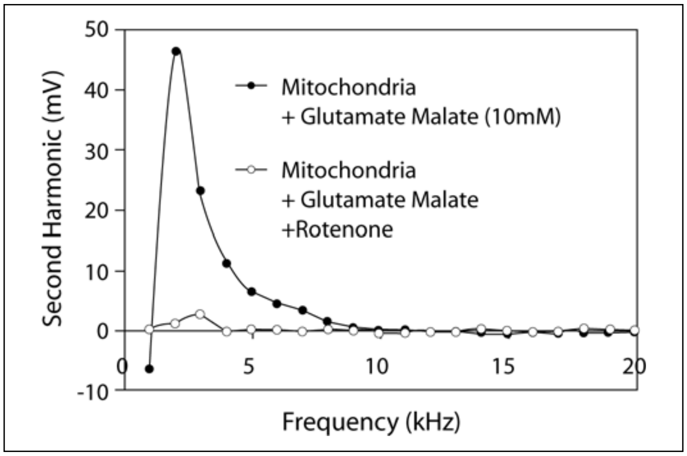


Figure 2. Second harmonic response vs. applied fundamental frequency of uncoupled mitochondria, in which the electron transport chain is active (closed circles, before adding the inhibitor rotenone) and inactive (open circles, after adding rotenone), for an applied field amplitude of 5 V/cm. In uncoupled mitochondria, the ATP synthase molecular turbine is inactive and a second, higher frequency (~12 kHz) peak, seen in budding yeast and *B. indicas*, is absent in this case.

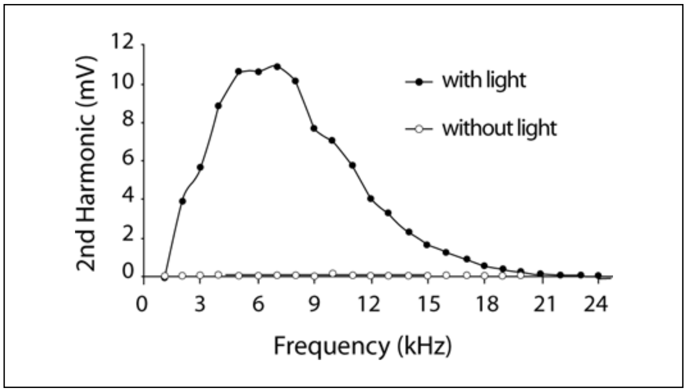


Figure 3. Second harmonic response vs. applied fundamental frequency for chloroplasts, with light and without light, for an applied field amplitude of 5 V/cm.

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INVERTED MICROSCOPE—Inside a copper low-level clean room to prevent contamination, Gustavo Cardena utilizes an inverted microscope—state-of-the-art equipment—which captures images from beneath. Cardenas, a doctoral student in physics, earned his baccalaureate degree at the Technology Institute of Monterey.

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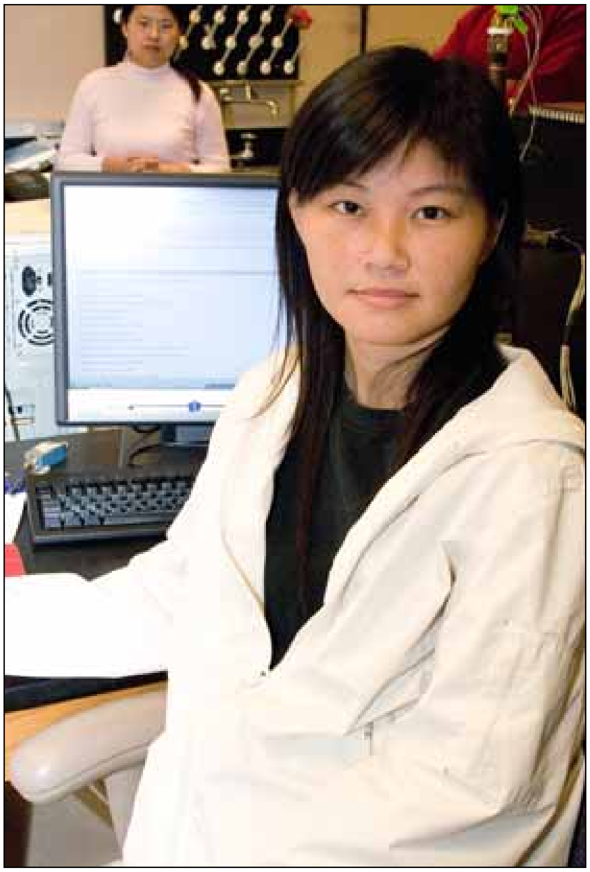
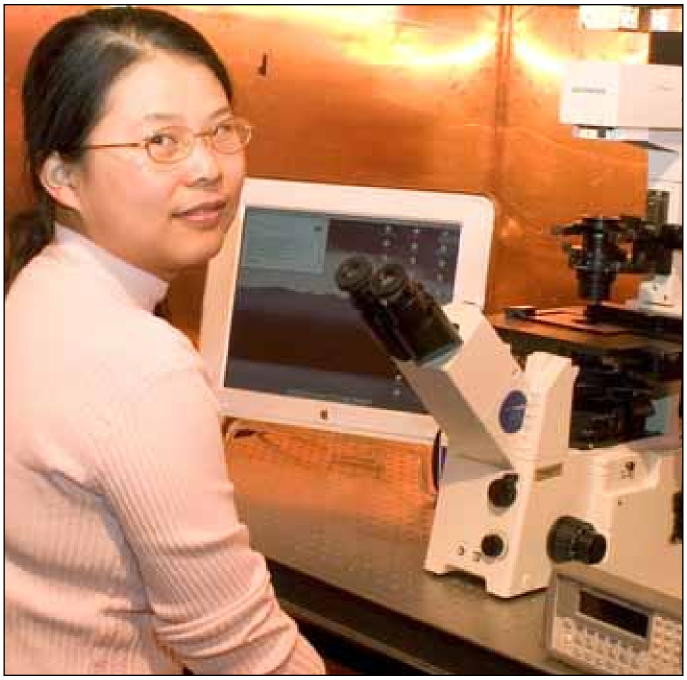
Miller, J. H., Jr., D. Nawarathna, V. Vajrala, J. Gardner, and W. R. Widger. "Electromagnetic Probes of Molecular Motors in the Electron Transport Chains of Mitochondria and Chloroplasts," *J. de Physique* IV (France) 131 (2005): 363-66.



PH.D. STUDENT—Shih-Ying Hsu earned her B.S. degree at the National Chung-Hsin University, China.

PUBLISHED STUDENTS—Students, such as Vijayanand Vajarala (*r*.) have the opportunity to publish their results in professional journals. Vajrala who earned his B.S. in physics at the University of Hyderabad, Indian, is now involved in doctoral study in physics at the University of Houston. Others involved in the study of liquid samples subjected to dielectric spectroscopy measurements are Dr. Dharmakeerthi Nawarathna (*l*.), from the University of Peradeniya, Sri Lanka, and Gustavo Cardenas (*kneeling*) from Monterey, a doctoral student in physics.



SUSPENSIONS—Jie Fang studies electromagnetic properties of live cells and proteins utililzing the inverted microscope. Engaged in doctoral study, she earned her baccalaureate degree at the Central China Normal University.

Nawarathna, D., J. H. Miller, Jr., J. R. Claycomb, G. Cardenas, and D. Warmflash. "Harmonic Response of Cellular Membrane Pumps to Low Frequency Electric Fields," *Phys. Rev. Lett.* 95 (2005): 158103–1-4.

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Vajrala, V., D. Nawarathna, J. R. Claycomb, and J. H. Miller, Jr. "Impedance Magnetocardiography Using High-T_C SQUIDs," IEEE *Trans. on Applied Superconductivity* 15.2 (2005): 680-83.

Presentations

Miller, J. H., Jr. "Condensed Matter Physics Phenomena in Biological Systems," Texas Center for Superconductivity, University of Houston, April 21, 2005. (*Invited.*)

—. "Electromagnetic Properties of Live Cells and Proteins," Los Alamos National Laboratory, National High Magnetic Field Laboratory, Los Alamos, NM, July 20, 2005. (*Invited.*)

—. "Electromagnetic Sensors of Nanoscale Biological Motors," Associated Nanotechnology Congress, Strategic Partnership for Research in Nanotechnology, 3rd Annual SPRING Conf., Rice U., Houston, TX, Oct. 10–11, 2005. (*Invited.*)

—. "Resonant Peaks in the Nonlinear Electromagnetic Responses of Live Cells and Mitochondria," Department of Bioengineering, Rice U., Houston, TX, Feb. 7, 2005. (*Invited*).

Miller, J. H., Jr., D. Nawarathna, H. Sanabria, V. Vajrala, and J. R. Claycomb. "Nonlinear Harmonic Responses of Live Cells Using High-T_c Superconducting Quantum Interference Devices," 24th Intl. Conf. on Low Temperature Physics, Orlando, FL, Aug. 10–17, 2005.

Miller, J. H., Jr., D. Nawarathna, V. Vajrala, J. Gardner, and W. R. Widger. "Electromagnetic Probes of Molecular Motors in the Electron Transport Chains of Mitochondria and Chloroplasts," Intl Workshop on Electronic Crystals, Cargèse, France, Aug. 21–27, 2005. (*Invited for expanded presentation.*)

Nawarathna, D., D. Warmflash, J. H. Miller, Jr., and J. Claycomb. "Noninvastive Probes of Mitochondrial Molecular Motors," *Bull. Am. Phys. Soc.* 50 (2005): 1342. Meeting of the American Physical Society, Los Angeles, CA, March 21–25, 2005.

Nawarathna, D., J. Gardner, G. Cardenas, J. R. Claycomb, J. H. Miller, Jr., and W. R. Widger. "Electromagnetic Probes of Molecular Motors in the Electron Transport Chains of Mitochondria and Chloroplasts," Associated Nanotechnology Congress, Strategic Partnership for Research in Nanotechnology, 3rd Annual SPRING Conf., Rice U., Houston, TX, Oct. 10–11, 2005.

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Funding and Proposals

Miller, J. H., Jr. "Dielectric Spectroscopy of Chemical and Biological Systems," Robert A. Welch Foundation, June 1, 2004–May 31, 2007, $165,000.

Miller, J. H., Jr. and J. Wosik. "Dielectric Spectroscopy of Biological Agents," DARPA/Naval Surface Warfare Center, Sept. 26, 2003–Sept. 25, 2005, $250,000.

Miller, J. H., Jr., D. S. McKay, G. E. Fox, J. Wosik, and D. Warmflash. "Biosensors Based on Dielectric Response: A Non-Geocentric Approach for *In Situ* Life Detection," NASA-Astrobiology Science and Technology for Exploring Planets Program. Requested for three years ($793,197 for UH, $102,080 for NASA-JSC), $895,277. (*Pending.*)

In 2006 we intend to submit at least one NIH (R01 or R21) proposal, likely in biomedical engineering or a related area, which will build on our recent results and publications on noninvasive electromagnetic biosensors.

UWB Tracking System Design with TDOA Algorithm for Space Applications

(Continued from page 22.)

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