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Probing Interactions at the Nanoscale: Sensing Protein Molecules and Protein Networks In Vivo Using On-Chip Electronic Nanosensors

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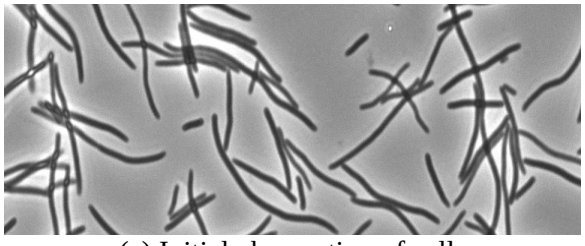
Looking at Live Cells by High-Resolution, High-Field NMR

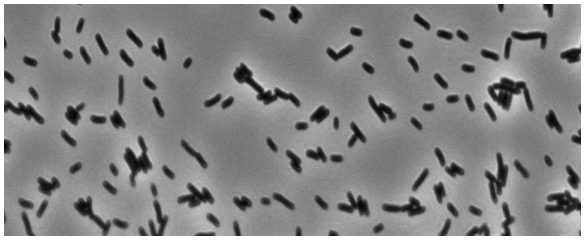
Cell metabolism can change drastically due to naturally occurring fluctuations in internal cell state and in environmental conditions. Genetic engineering also often results in modifications to cell metabolism in potentially undesired and unanticipated ways that can lead to cell stress, loss of function, and loss of viability. An important component of Systems Biology is to gain an understanding of such changes and their relationships to the dynamics of genetic and protein networks. The main requirement for performing such studies is the ability to probe the state of the cells. However, existing approaches to probing cell state suffer from a variety of limitations since no current approach can examine the dynamic state of a large number of cell components *simultaneously*, in *real-time*, within *living cells*. We are now exploring the use of NMR for such purposes.

Nuclear magnetic resonance (NMR) is a quantitative non-destructive technique reporting at the molecular level. As such, it may be very informative about differential cell biochemistry even if the changes have not reached the level of observable differentiation in the phenotype. We have started an NMR study to distinguish and characterize intact, live cells of various behavior and origin, as well as looking at carefully prepared cell extracts, using both more conventional tube-based, as well as capillary flow NMR techniques for very small quantity samples.

Our pilot studies have been conducted on selected bacterial cell cultures. We transformed *Escherichia coli* cells with a plasmid that overexpresses a recombinant protein, the Yellow Fluorescent Protein. This overexpression likely places a metabolic stress on the cells, and they become filamentous after several hours of growth, as seen in the microscope image in Figure 1(a). After additional growth, the cells return to their normal rod shape, as can be seen in Figure 1(b).



(a) Initial observation of cells



(b) Same cells after 4 hours

Figure 1: Filamentous cells which then mutate to normal looking cells

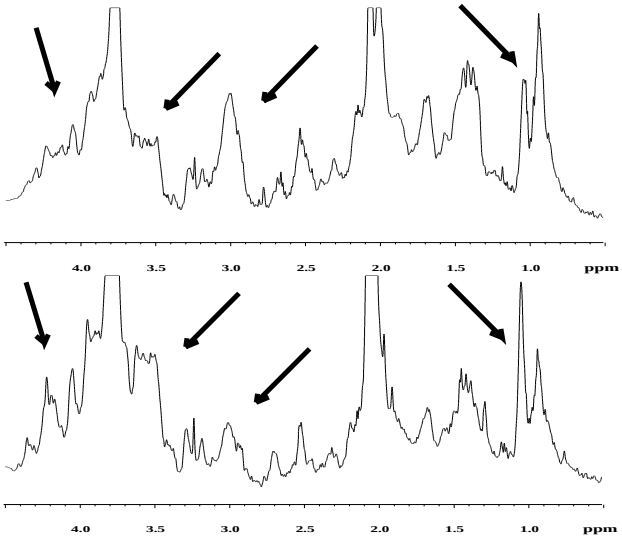


Figure 2: Representative ^{1}H-NMR spectral segments of cell samples with marked differences

We have conducted several consecutive experiments on various cell samples. Simple ^{1}H NMR spectra show specific changes between samples (representative segments are shown on Figure 2), which seem to be diagnostically correlated with differential origin and state of these cells, which we'll demonstrate on the poster. Cell extracts are also prepared with as little biochemical or chemical bias introduced as possible. Such cell extracts can be studied at very low absolute amounts also taking advantage of capillary flow NMR. We'll also discuss possible future extensions of such NMR studies.

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A NOVEL HIGH-FREQUENCY SENSOR FOR BIOLOGICAL DISCRIMINATION

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ABSTRACT

We have developed a high-frequency electronic biosensor for probing molecules and cells. We use microfluidic tectonics (μFT) to create a 3-dimensional parallel-plate device that results in enhanced electronic coupling to sample volumes as small as picoliters over a frequency range of 0.05–40 GHz. In this frequency range, we are able to access two distinct regimes, α- and β-dispersion. The former corresponds to permittivity enhancement due to rearrangement of small ions, including screening at the fluid interface, and the latter arises from distortions of cellular membranes and macromolecules. Here we report experiments differentiating polymerase chain reaction (PCR) products before and after amplification cycling.

Keywords: dielectric spectroscopy, microfluidics, electronic biosensing

1. INTRODUCTION

The need for rapid characterization of molecular and biological materials is extremely important for research, clinical, and defense applications. Most current optical and chemical techniques are effective for analyzing such specimen [1–3]; however, they often require chemical modification. Furthermore, they can be time-constrained due to, for example, photobleaching of fluorophores Recently, purely electronic techniques such as capacitance cytometry [4] and microwave spectroscopy [5–8], as well as fourier-infrared spectroscopy [9,10], have offered viable solutions to these issues whilst probing a range of length and time scales for very small sample volumes.

In this report, we discuss our development of a microwave-frequency-based biosensor of parallel-plate geometry that is embedded within a microfluidic device. This biosensor allows us to perform dielectric spectroscopy on a variety of biological samples—from cells to molecules—in solution. Because the device is purely electronic, the sensor allows for rapid characterization with little sample preparation or chemical alteration. In addition, this biosensor is capable of probing length scales from millimeters to microns over a frequency range 50 MHz to 40 GHz, and sample volumes as little as picoliters [4,5]. Our high-frequency biosensor has evolved from previous device designs based on a coplanar waveguide (CPW) geometry, where we measured changes within the plane of the CPW device [5]. For our current device, we extend the center conductor of the CPW and employ microfluidic tectonics (μFT) [11] to embed two CPW devices within a

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microfluidic channel located between the two overlapping extended conductors. The out-of-plane capacitive coupling between the two conductors is greater than in our previous CPW designs and more importantly, leads to an enhanced sensitivity. For example, at 40 GHz, with deionized (DI) water in the fluidic channel, the CPW device had insertion losses of -30 dB, while the new parallel-plate design has only -20 dB. Our utilization of μFT allows us to incorporate this high-frequency electronic biosensor with a variety of lab-on-a-chip architectures.

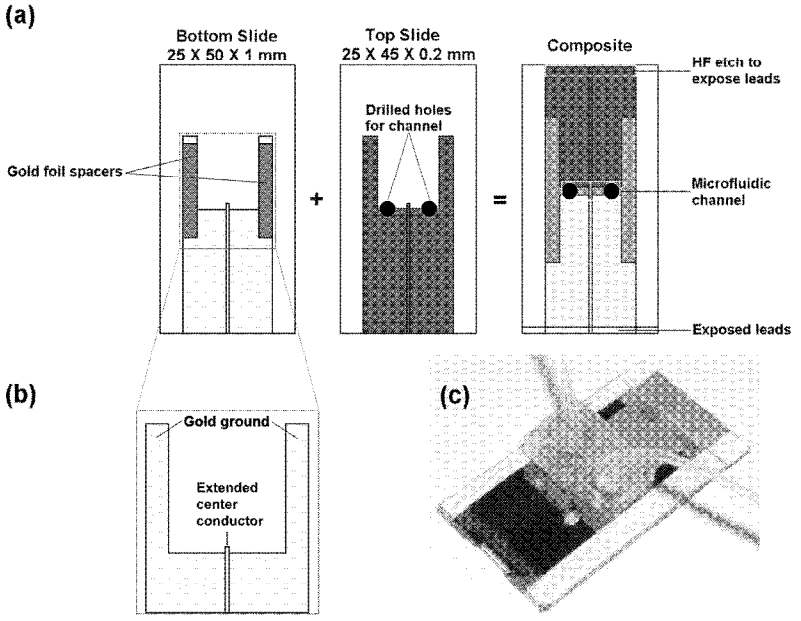

Figure 1: The high-frequency electronic biosensor we have developed. (a) Microfluidic tectonics is used to create a microfluidic channel perpendicular to and between the two CPW extended center conductors previously deposited on glass slides. The microfluidic channel height is defined by the gold foil spacer and ranges from 5 to 100 μm; (b) An expanded view of CPW with extended center conductor. The center conductor is 80 μm wide and extends 300 μm beyond the ground planes. The ground planes are 20 μm from the center conductor. The top and bottom grounds overlap ≤ 200 μm; (c) The completed device is symmetric and fluid may flow in either direction.

2. EXPERIMENTAL

Figure 1 is a schematic of our high-frequency electronic biosensor. We fabricate this sensor by first depositing a 50/500 Å seed layer of Ti/Au onto two glass microscope slides. We then use photolithography to pattern the gold. The exposed gold is

subsequently electroplated to a thickness of 4-6 μm. After reactive-ion-etching the remaining photoresist and removing both the unplated gold with a standard iodine-based gold etchant and the Ti with a buffered-oxide etchant, we deposit a 1000 Å layer of Si_3N_4 to the region where the fluidic channel will be defined. This is done so as to decrease the adhesion of biological materials to the gold. Under an optical microscope, we align the two slides such that the center conductors overlap one another in a parallel-plate geometry (overlap region is 80 μm x 500 μm). We control the separation between the two CPW devices using gold foil spacers 3–25 μm thick. The foil additionally ensures good coupling between the grounds of both slides. Following alignment, we employ μFT to create a microfluidic channel running perpendicular to the overlapping center conductors while simultaneously bonding the slides together (see Figure 1). We complete the device by inserting 0.02" ID vinyl tubing through pre-drilled input and output holes of the device [11]. All of our devices are designed to have a 50 Ω matched impedance and minimal insertion loss for 0.05–40 GHz. With these characteristics, we can expect a sensitivity of 0.05 dB.

3. RESULTS AND DISCUSSION

At low frequencies (≤ 1 GHz), we can observe ionic rearrangement and screening (α-dispersion). At high frequencies (> 20 GHz), we can access the β-dispersion regime and probe unique macroscopic distortion modes of biomacromolecules. While not exactly zero, the dipole moment of water is rapidly decreasing at these high frequencies, and consequently, we can expect β-dispersion to be the most significant contribution to our obtained spectra.

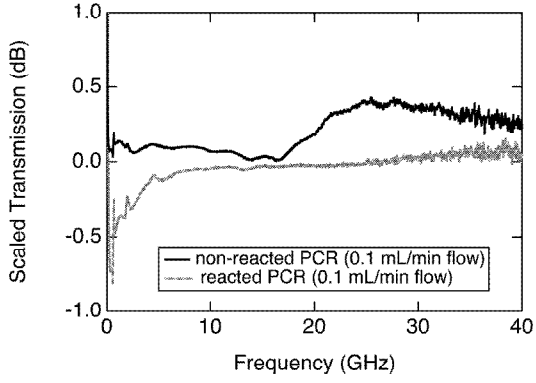

Figure 2: Transmission of non-reacted PCR primers and reacted PCR products scaled to DI water. The spectra correspond to dynamic fluid flow through the microfluidic channel at 0.1 mL/min.

As a first demonstration of our device, we have measured PCR products. We are able to distinguish between non-reacted primers for PCR amplification and reacted PCR products (24 amplification cycles). Figure 2 shows representative transmission spectra of the two different DNA solutions obtained from a single device and scaled to DI water. We have obtained similar spectral features from additional devices and are currently developing a quantitative model to explain our results. As mentioned, the low frequency domain shows large contrast to DI water due to ionic concentrations of the solutions. As we have reported previously [5], solutions of DNA show little difference to the spectra of DI water at higher frequencies, as seen in the reacted PCR products. Above 20 GHz, both reacted and unreacted PCR products have a corresponding onset of interference fringes in the reflection spectra indicating the possibility of resonant absorption for this frequency range. This initial demonstration of molecular differentiation using a high-frequency electronic biosensor shows the great promise of *electronic biosensing*.

4. CONCLUSIONS

Electronic sensors for biological applications provide fast quantitative results without the need for chemical alteration. Here we have shown that similar biological solutions are distinguishable using our parallel-plate geometry for dielectric spectroscopy. Understanding these results in terms of a quantitative model is still an active pursuit; however, these results can be understood by simple qualitative behaviors.

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