Towards a biohybrid sensing platform built on impedance-based bacterial flagellar motor tachometry

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Abstract—Though the chemotaxis sensing system of *Escherichia coli* is known to approach fundamental biosensing limits, few attempts have been made to co-opt it as the front end for a biohybrid sensor. We propose a biohybrid sensor that electrochemically monitors chemotactic bacterial flagellar motor (BFM) rotation speed and direction to infer analyte concentration for a low-power, fast, and sensitive response. We first present the design and fabrication of a four point impedimetric array that uses current injection electrodes to circumvent electrode polarization screening, enabling solution resistance monitoring within a four-micron by four-micron region. We then demonstrate the first lithographically patterned silica shaft encoders for the BFM, which utilize localized biotin-avidin chemistry to selectively bind to the BFM and encode rotation. When these two components are integrated by bringing the rotating encoders in proximity with the microelectrodes, they will comprise an electrochemical method for observing the BFM. Such an impedance-based biohybrid sensor obviates the need for a microscope and in principle may be multiplexed and scaled to large arrays of bacterial cells, enabling the development of deployable low-power and fast sensing systems that directly observe the BFM to infer analyte concentration.

I. INTRODUCTION

Growing interest in mobile aquatic microbotics systems has increased demand for real-time, sensitive, and low-power biosensors [1], and electrochemical sensor platforms have been developed [2]. The chemotaxis biosensing system in *Escherichia coli* detects a wide variety of analytes and is known to approach fundamental physical constraints set by diffusion noise on speed and sensitivity given the 1 fL volume of the cell [3]. The detection limit for L-aspartate is 3.2 nM, which corresponds to the detection of approximately three molecules per cell volume [3], while large step changes in L-serine affect motility within 300 ms [4].

The bacterial flagellar motor (BFM) of *E. coli* and other bacteria is one of few naturally occurring nanoscale rotary motors [5]. The BFM (illustrated in Fig. 1a) drives cell propulsion through drag-dominated microscale fluid environments. It consists of a basal rotary motor roughly 50 nm in diameter, a flexible hook joint, and a thin filamentous flagellum up to 10 µm long [5]. The chemotaxis system monitors chemoreceptor-analyte binding and makes real-time decisions about which direction to rotate the BFM to bias movement towards nutrients and from toxins [6].

Despite this natural capacity, no modern engineered biosensing system approaches the time-sensitivity limits within the same volume as a cell. Biosensors with a detection limit of 1 fM or lower, such as the enzyme-linked immunosorbent assay (ELISA), generally rely on label-enhanced capture methods which require incubation times on the order of 30 min or longer [7]. Although commercially available surface plasmon resonance sensors have a detection limit and response time comparable to *E. coli*, they are bulky instruments at least 18 orders of magnitude larger than a bacterium [8].

There is an opportunity to use bacteria as the front-end for a biohybrid sensor with a fast response, but this requires real-time BFM observation. Moreover, relatively few studies have used BFM motor behavior as the front end for a biohybrid sensor. These studies have been exclusively limited to microscopy experiments [9] since established methods for observing the BFM (tethering spherical beads to flagellar hooks [10] and tethering cells by a flagellar stub to observe body rotation [11]) require the use of a microscope and therefore do not meet the power and size constraints for biohybrid sensing platforms. A means for observing the BFM without a microscope is currently not available.

We propose a biohybrid sensor that monitors BFM rotation electrochemically to infer analyte concentration for a low-power, fast, and sensitive response. Two challenges must be overcome to achieve our vision as pictured in Fig. 1b-c: localized monitoring of solution impedance and enhancing the impedance signal of a rotating BFM with a dielectric shaft encoder. In this paper, we present the fabrication of four-point impedimetric microelectrode arrays that monitor solution impedance perturbations in a 4 µm by 4 µm square region.
We also introduce the first photolithographically patterned silica shaft encoder labels the BFM via avidin-biotin chemistry. A dielectric bead that displaces saline solution concentration to changes in motor direction and speed. (b) A lithographically patterned silica shaft encoder labels the BFM via avidin-biotin chemistry. (c) The encoder rotates in close proximity to a pair of microelectrodes that continuously monitors the solution impedance as the encoder perturbs the electric field between them. (d) Equivalent circuit model of bead detection including the interface circuit.

II. A MICROELECTRODE ARRAY DETECTS LOCALIZED IMPEDANCE CHANGES

A. Equivalent circuit model for dielectric bead detection

The equivalent circuit model for the microelectrode measurements is shown in Fig. 1d. For this model, we consider the detection of a dielectric bead by a pair of 4 μm by 4 μm potential sense electrodes and a pair of 100 μm by 100 μm current injection electrodes immersed in saline solution. Electrode polarization gives rise to large double layer constant phase element impedances $Q_v$ and $Q_i$ that are bypassed at higher frequencies [12]. The larger size of the current injection electrodes ensures that the interface impedance is below the solution resistance at approximately 10 kHz, enabling solution resistance measurement above that frequency. A large input impedance instrumentation amplifier minimizes the effects of parasitic capacitance on sense electrode measurement according to Linderholm et al. [13] and interfaces the device with an E4980AL LCR meter (Keysight Technologies, Santa Rosa, CA). A dielectric bead that displaces saline solution between the sense electrodes increases measured solution resistance by the Coulter counter principle [14].

Impedance spectra in Fig. 3 measured for the array immersed in aqueous KCl varying from 1 mM to 100 mM show that solution conductivity is discernible between 10 kHz to 100 kHz. As expected, decreasing solution impedance is observed with increasing KCl concentration. The ideal phase angle for maximum sensitivity is 0°, since this would be a...
Fig. 3. Impedance spectra for the electrode array immersed in the following solutions: deionized water (blue circles), 1 mM KCl (red diamonds), 10 mM KCl (yellow triangles), and 100 mM KCl (purple crosses). A 200 mV amplitude stimulus is used.

Fig. 4. Correlation of impedance signal to bead pass. (a) Bright field view of electrode array as 2 μm diameter fluorescently labeled polystyrene beads pass over. Scale bar is 10 μm. (b) The resulting synchronized fluorescence and impedance measurements. The fluorescence signal (blue; λ exc = 495 nm, λ em = 519 nm) was summed in the blue square region between the electrodes as indicated in (a). The 10 kHz impedance signal (red) was processed by a DC notch filter to remove drift.

Direct measurement of solution resistance. The measured phase angle is between $-25^\circ$ and $-5^\circ$ from 10 kHz to 100 kHz for 1 mM to 10 mM KCl, which is close to the ideal. The 100 mM KCl solution resistance is below 1 kΩ, so that double layer impedance dominates the response until 100 kHz, where poles from the instrumentation amplifier begin to dominate. Therefore, the solution’s ionic strength should be kept between 1 mM to 10 mM for resistance sensing.

D. Array detects conductivity changes caused by spherical dielectric microbeads with high spatio-temporal resolution

Representative results from the synchronized optical and 10 kHz impedance measurements in Fig. 4 demonstrate detection of 2 μm diameter fluorescent polystyrene beads as they flow across the array with good temporal agreement. The intensity of the fluorescence signal within the 4 μm by 4 μm region between the potential sense electrodes is co-plotted with the measured impedance change. Each bead caused an increase in impedance as it passed over the electrode array, with an SNR of 7 dB for the strongest impedance changes. The height of the bead relative to the electrodes was not well-resolved by the long working distance objective and could have varied from 1 μm to 10 μm. However, higher bead passes generally resulted in less significant impedance changes.

Beads that did not pass directly over the sense electrodes were not detected, suggesting spatial resolution within 4 μm. The impedance measurements were sampled at a rate of 37 Hz, which would be sufficient to resolve a four-armed shaft encoder rotating at 4.6 Hz or slower.

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III. A MICROFABRICATED BFMI SHAFT ENCODER

A. Encoder fabrication and avidin functionalization

The encoder fabrication process is presented in Fig. 5 and takes place on 675 nm thick 6° diameter Si wafers. First, a 400 nm sacrificial layer of Ge is deposited by low pressure chemical vapor deposition (LPCVD) [15], immediately followed by 650 nm of low temperature oxide (LTO) by LPCVD. Second, a photoresist mask is lithographically patterned and UV hard baked. $4 \times 10^6$ beads fit onto 1 cm$^2$, yielding 5 x $10^8$ encoders per wafer. Third, LTO is etched in a reactive-ion etcher to define the encoders. Fourth, a layer of 5 nm Ti and 30 nm Au patches are patterned in the center of each encoder using EBPVD and lift-off. Finally, the wafer is immersed in 30% hydrogen peroxide for 2 h at 23°C to dissolve the Ge sacrificial layer and release the encoders. Deionized water is then sprayed on the surface of the wafer to remove the encoders and collect them in a centrifuge tube.

The encoders are centrifuged at 4000 rcf for 10 min, resuspended in 1 mL ethanol (EtOH), and washed 3 times in EtOH. Then thiol-PEG-biotin (MW2000, Laszys Biosciences, Arab, Al) is dissolved in EtOH to 10 mM and incubated for 2 h at 23°C to 100 μL of the encoder suspension for thiolation. This reaction is agitated 12 h at 150 rpm at room temperature. Afterwards, the suspension is washed twice in EtOH and resuspended in 100 μL phosphate buffered saline (PBS, pH 7.2), and 0.1% Tween-20. Neutravidin is added to 10 μg mL$^{-1}$ and incubated 20 min with 150 rpm shaking. Lastly, the encoders are washed 3 times and resuspended in 150 μL PBS.

B. Strain and growth conditions

The E. coli strain used in this experiment was derived from MTB32 which suppresses the flagellar filament and expresses a biotin accepting peptide sequence on the flagellar hook [10]. Standard lambda red recombination [16] was used to knock out the CheY gene in MTB32, conferring a straight-swimming mutant with kanamycin antibiotic resistance (final genotype: ΔfliC, ΔCheY::KanR, flgE-BAP).

These cells are grown from stationary culture in 3 mL T-broth (1% w/v tryptone, 0.5% w/v NaCl) at 30°C with 250 rpm shaking to an optical density at 600 nm of 0.4. Then the cells are spun in a centrifuge at 1000 rcf for 1 min and washed twice in motility medium (MtMd: 10 mM KPO$_4$, 0.1 mM EDTA, 1 mM L-methionine, pH 7.0). Cell hooks are then biotinylated by resuspension in 80 μL of MtMd, 10 μL of BiomixA (0.5 M bicine buffer, pH 8.3), 10 μL

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Fig. 5. Encoder fabrication and tethering process: (i) Deposition of Ge and low temperature oxide (LTO) by LPCVD. (ii) Patterning and plasma etch of encoders. (iii) Lift-off patterning of Ti/Au patches on the center of each encoder. (iv) Release in 30% hydrogen peroxide. (v) Avidin (AV) functionalization via thiol-PEG-biotin (SH-P-B) conjugation to Au surfaces. (vi) Tethering to biotinylated hooks on immobilized cells.
µ would be sufficiently slow for the electrode array to detect with 10\(^2\) min. Neutravidin-AlexaFluor647 is immobilized in the center (red, \(\lambda_{ex} = 651\) nm, \(\lambda_{em} = 667\) nm). (b) Confocal microscope image of biotinylated cell hooks. Bacteria were stained by DAPI (blue, \(\lambda_{ex} = 358\) nm, \(\lambda_{em} = 461\) nm) and their biotinylated hooks were tagged by neutravidin-AlexaFluor647 (red). (c) Bright field time lapse of BFM-driven clockwise encoder rotation across 400 ms. Frames are separated by 100 ms.

C. Biotin functionalization enables defined attachment of silica shaft encoders to BFM

Biotin is localized to the center of each shaft encoder and to the flagellar hooks as visualized by fluorescently labeled neutravidin in 6a-b. The tight interlayer alignment is achieved by patterning 500 nm diameter gold patches in the center of each encoder via stepper lithography. Defined surface functionalization allows precise control over where each encoder is tethered and controls the load on the motor, although the efficiency of the functionalization is low and needs further optimization. Using the hook instead of the flagellar filament ensures more control over the length of the tether and consistency in the loading of the BFM.

Functionaization of the hooks and encoders resulted in BFM-driven rotation of silica encoders 4 \(\mu\)m in diameter at a frequency of 2 Hz as shown in the time lapse in Fig. 6c. This rotation rate is similar to the rotation of motors loaded by spherical beads reported in the literature [10] and should have a minor effect on the chemotactic sensing response. This would be sufficiently slow for the electrode array to detect each pass of the encoder’s four arms.

IV. CONCLUSION AND FUTURE WORK

Towards developing a biohybrid sensor we have: fabricated and tested a microarray electrodes to measure solution impedance in a 16 \(\mu\)m\(^2\) area; fabricated and functionalized silica beads as shaft encoders for bacterial flaggella. Beyond biosensing, our micropatterned shaft encoders can improve existing optical BFM observation methods. The loading on the cell can be controlled by the size of the encoder and the position of the tether, enabling more uniform speeds between BFMs. Consistent speed measurements would also enable a proxy measurement of the proton motive force (PMF), a proton separation across the inner membrane that is of metabolic importance to \(E.\) coli and proportional to motor speed. Real-time PMF measurements currently require the use of sensitive fluorescence microscopy [17]. A method for tachometry could eventually enable closed loop control for BFM microactuation.

When completely integrated, these techniques would comprise a fast-response biohybrid sensor that uses chemotactic \(E.\) coli as the detection element while low-power electrochemical measurements are used to read out the motor state. An impedance-based measurement could in principle be scaled up to large ensembles of BFMs and enable deployable aqueous biohybrid sensing applications.

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