Microwave frequency sensor for detection of biological cells in microfluidic channels

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We present details of an apparatus for capacitive detection of biomaterials in microfluidic channels operating at microwave frequencies where dielectric effects due to interfacial polarization are minimal. A circuit model is presented, which can be used to adapt this detection system for use in other microfluidic applications and to identify ones where it would not be suitable. The detection system is based on a microwave coupled transmission line resonator integrated into an interferometer. At 1.5 GHz the system is capable of detecting changes in capacitance of 650 zF with a 50 Hz bandwidth. This system is well suited to the detection of biomaterials in a variety of suspending fluids, including phosphate-buffered saline. Applications involving both model particles (polystyrene microspheres) and living cells—baker's yeast (*Saccharomyces cerevisiae*) and Chinese hamster ovary cells—are presented. © 2009 American Institute of Physics. [DOI: [10.1063/1.3187149](https://doi.org/10.1063/1.3187149)]

I. MOTIVATION

Electrical properties of living systems are of rapidly expanding interest in medical and biophysical research. Rapidly growing field of microfluidics offers a possibility of nondestructive cell analysis and is especially applicable to single-cell measurements (see Ref. 1 and references therein). Suspensions of cells can be pumped through microfluidic channels equipped with different configurations of electrodes that provide tailored nonuniform fields.^{2–5} Response of suspended bioparticles to the field is utilized to detect, sort, or manipulate them for diagnostic and research purposes.

The flow of biological materials in microfluidic channels through regions of nonuniform field induces changes in capacitance near electrodes. Cells can be modeled as parallel circuits with capacitance and variable resistance, and the resulting change in impedance can be measured at different frequencies using electronic detectors. Unlike the classical analytical techniques, such impedance measurements can be performed in a way that does not disrupt the cellular membrane or complex environment and mechanisms within. This allows studies of cell composition, cycles, and kinetics on a wide variety of eukaryotic cells, from micro-organisms such as yeast to complex mammalian and, in particular, human cells. Furthermore, cells can be sampled and analyzed one at a time, with the ability to circulate many cells past the electrodes in a short period of time.^{6,7}

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Diverse applications include differentiation between subpopulations of cells based on pathological stages in development,² detection of blocked ionic channels,^{3,8} or quantitative measurements of DNA contents⁹ that enable identification of genetic abnormalities.

While it is straightforward to detect the presence of the biomaterials in the channel, it is not always easy to discriminate between different types of bioparticles or between differences in size and structure of the same type. For example, the dielectric properties of biological cells vary dramatically over many parameters (volume, shape, composition, etc.) between 5 kHz and 200 MHz,¹⁰ making it difficult to choose a frequency that would depend on a single parameter change.¹¹ In addition, in some cases the effects of the field can damage biological materials and alter their properties.^{12–18} These undesirable outcomes can be eliminated or greatly reduced if the sensing is performed at higher gigahertz frequencies, where capacitance sensitivity tends to increase.¹⁹

Detection systems based on flow impedance measurements^{20,21} have several advantages over the more traditional optical methods.^{22,23} They are highly sensitive, easy to set up and control, and allow high throughput screening and analysis of cellular properties and function using sample volumes on the order of microliters. Moreover, they offer a possibility of electronic integration not accessible with corresponding optical detectors. In this paper we focus on an all-electronic system that operates at 1.5–2 GHz. The particular geometry of our system allows cells to flow over coplanar electrodes embedded in the bottom of a channel. We then sense the change in capacitance of the volume directly above the electrodes.

II. INDUCED CAPACITANCE CHANGE—THEORETICAL BACKGROUND

Assume that the capacitance due to a region of nonuniform electric field within a microfluidic channel filled with dielectric of permittivity ϵ_m is equivalent to C_0 . The stored energy of this region can then be written as

$$W_0 = -\frac{1}{2}C_0U^2, \quad (1)$$

where U is the potential applied to the electrodes. When a particle of permittivity $\epsilon_p(\omega)$ is placed within this region, the capacitance of the resulting system changes to C , and the new free energy is

$$W = -\frac{1}{2}CU^2 = -\frac{1}{2}C_0U^2 - \frac{1}{2}\int_{\xi} \mathbf{E} \cdot \mathbf{P} d\xi. \quad (2)$$

Here, $\mathbf{E}$ is the applied nonuniform electric field and $\mathbf{P}$ is the polarization of the particle due to this field.^{24,25} The integral in the above equation is over the volume ξ of the entire region; however, the induced polarization is zero everywhere except in the region occupied by the particle ξ_p . Assuming the case of a lossless dielectric particle in a lossless dielectric medium, polarization can be written as

$$\mathbf{P} = (\epsilon_p - \epsilon_m)\mathbf{E}_i, \quad (3)$$

with $\mathbf{E}_i$ the field inside the particle resulting from the separation of charges and is generally obtained as a solution of the Laplace equation $\nabla \cdot (\epsilon_p \nabla \phi) = 0$ with appropriate boundary conditions, where ϕ is the field potential at any point in the region ξ_p . If we assume that the particle is spherical, with radius a small enough so that the changes in electric field or potential are not significant over the volume ξ_p , this field can be approximated as (see, e.g., Refs. 24 and 25)

$$\mathbf{E}_i = \frac{3\epsilon_m}{\epsilon_p + 2\epsilon_m}\mathbf{E}. \quad (4)$$

The induced change in capacitance, $\Delta C = C - C_0$, is then found to be

$$\Delta C = 4\pi\epsilon_m a^3 K_{\text{CM}} \frac{|\mathbf{E}_{\text{rms}}|^2}{U^2}. \quad (5)$$

The quantity K_{CM} is a Clausius–Mossotti factor, and it determines the response of a dielectric particle immersed in another dielectric medium to an applied field. Specifically, for particles in ac fields, it depends on the frequency of the applied field through a combination of dielectric permittivities of the particle and the medium. The exact form of $K_{\text{CM}}(\omega)$ varies with geometry and symmetry of a particle; for a sphere of permittivity $\tilde{\epsilon}_p(\omega)$ embedded in a medium of permittivity $\tilde{\epsilon}_m(\omega)$, it is given by

$$K_{\text{CM}}(\omega) = \frac{\tilde{\epsilon}_p(\omega) - \tilde{\epsilon}_m(\omega)}{\tilde{\epsilon}_p(\omega) + 2\tilde{\epsilon}_m(\omega)}, \quad (6)$$

where notation $\tilde{\epsilon}$ denotes complex permittivity

$$\tilde{\epsilon}_i(\omega) = \epsilon_i - j \frac{\sigma_i}{\omega}. \quad (7)$$

In the above, ϵ_i represents a dielectric constant, and σ_i is an effective conductivity of the material i ; ω denotes frequency, while $j = \sqrt{-1}$. For low-loss dielectrics $\sigma_i/\omega\epsilon_i \ll 1$ (e.g., very low conductivities or high frequencies; see Ref. 26) so that we can assume that $\tilde{\epsilon}_i \approx \epsilon_i$.²⁷ The sign of K_{CM} determines whether a particle polarizes more or less than the surrounding medium.

Interfacial polarization of a biological cell, resulting from the membrane-trapped charged particles, dominates all the other types of polarization within the region of 5 kHz–200 MHz.¹⁰ Since the ability of charged particles to follow changes of the field varies with frequency, we can expect that the Clausius–Mossotti factor changes dramatically in this interval.¹¹ These changes are specific to each type of cell providing the Clausius–Mossotti factor with a role of a “dielectric phenotype.” This is useful in applications that separate or sort out cells with very different phenotypes. On the other hand, because of this sensitivity in this frequency interval, even small changes in cell size and position in the channel (in an inhomogeneous field) can impact dielectric phenotype heavily in this interval, making it problematic to choose a frequency bandwidth with clear dependence on a single parameter and to obtain consistent normalization for size.¹¹ This is why we prefer to work in the gigahertz frequency region, where such effects are minimized and the signal can be related primarily to changes in capacitance.

III. DESCRIPTION OF THE APPARATUS

A. Overview of the detection system and equipment

The detection system we have used is represented schematically in Fig. 1. The signal from a rf source (gigahertz range and modulated at 50–100 kHz) is split between an unchanging reference path and a sensing path; the latter contains a quarter-wavelength resonant sensor connected to the microfluidic electrode array. In the present example, when the sensor is not coupled to the electrodes spanning the microfluidic channel, its resonance frequency is around 1.8 GHz. After connection is made to the microfluidic channel, the additional capacitance results in a shift in the resonant frequency to about 1.5 GHz, and the insertion phase of the resonator changes. The reference and the sensing paths are recombined using a mixer to form a microwave interferometer. Once adjusted properly and operating at the resonant frequency, any difference in phase between the paths produces a signal that can be related to the change in capacitance, ΔC , as given by Eq. (5).

Because the signal is small and easily obscured by the noise, it is sent to a lock-in amplifier (LIA), where a narrow-band detection of the mean output from mixer is made possible. During the operation, the rf frequency is set near the resonant frequency f_0 , and the delay line is adjusted to

FIG. 1. Zeptofarad detection system. Signal from the source is split in two paths, reference and sensing. Between points 1 and 2, sensing path contains the resonator sensor coupled to the microfluidic device. V_{res} is the amplified signal out of the sensor and recombined with V_{ref} using a mixer. The resonator is the center transmission line, labeled C. Its open-circuit end is coupled to microelectrodes via brass wires, which contact wider electrodes, before the microchannel electrodes, as shown. The capacitor at the other end of the resonator acts as a rf short-circuit. In this figure, we also show a general scheme with a port for applying low frequency DEP signals for cell actuation (V_{DEP}); however, we do not discuss cell actuation in this paper (interested readers, see Ref. 20). The region of the microchannel is enlarged to show three interdigitated electrodes extending across the channel in a direction perpendicular to the flow. The wider contact electrodes are at the top of this photomicrograph.

null the output (i.e., so that the nominal phase between the two paths, θ , is $\pi/2$). In this manner, a small change in capacitance, ΔC , produces a detectable signal, $S \approx G|V_{\text{ref}}||V_{\text{res}}|\cos \theta$, which, for $\theta = \pi/2 + \varphi$ and φ very small, we can approximate by

$$S \approx G|V_{\text{ref}}||V_{\text{res}}|\left(\frac{\partial \varphi}{\partial C}\right)\bigg|_{f_0} \Delta C. \quad (8)$$

In the above equation, G represents the mixer-and-LIA gain, V_{ref} and V_{res} are the signals from reference and resonance paths, respectively, and φ is the phase difference induced by ΔC . The term $(\partial \varphi / \partial C)|_{f_0}$ gives the rate of change of phase with capacitance near the resonant frequency. We discuss how to estimate this in the Appendix. Capacitance ΔC can be using Eq. (5) and from simulations of the field due to the microelectrode geometry.²⁰

B. Sensor

The sensing part of our apparatus consists of a quarter-wavelength resonator coupled to electrodes in a microfluidic channel. The microfluidic device used in this work was fabricated using a commercial foundry. The device is often glued onto a Plexiglas block for mounting. The channel has a (roughly) trapezoidal cross section; it is 40 μm deep, with widths on the top and bottom approximately 40 and 120 μm , respectively. The interdigitated microelectrodes [pictured in the photomicrograph in Fig. 2(b)] extend completely across the bottom of the channel. They are made of 180 nm thick platinum on a 20 nm tantalum adhesion layer. Microelectrodes are 25 μm wide with a 25 μm gap in between each pair (different microelectrode configurations—i.e., with one or more gaps—can be fabricated within the same channel.) They are connected to the wider long electrodes [see Fig. 2(a)] and contact pads outside the microfluidic channel region, where we

FIG. 2. (a) Electrodes for coupling the resonator to the microchannel viewed from below and shown extending into three interdigitated microelectrodes. Brass wires come into contact with the pads on the left. Total length of the electrodes is 1.5 mm, and about half is covered by glass (dark area to the right in the background). (b) Interdigitated microelectrodes (five in this example) viewed from above and through the channel. They are 25 μm wide, with 25 μm gaps. Light refraction makes the walls of the trapezoidal shaped channel cross section appear as dark strips in the micrograph.

connect the resonator, as will be explained. The long electrodes are 100 μm wide and 1.5 mm long, with half of that length covered by glass as the electrodes enter the channel region of the sensor. The electrodes are wider outside of the channel to reduce electrical losses at gigahertz frequencies.

The resonator-based sensor is located between points 1 and 2 in Fig. 1. The transmission line in the center is a quarter-wavelength resonator. It is edge-coupled on either side to short-circuited quarter-wavelength input/output signal lines (these can be used interchangeably because of the symmetry). The coupled line resonator is fabricated using grounded *Rogers Cu-clad 5880* dielectric substrate, with thickness of $h=1.5$ mm and relative dielectric constant of $\epsilon_r=2.54$. Characteristic impedances are $Z_{0,\text{in/out}}=50$ Ω for each of the two input/output lines and $Z_0=90$ Ω for the center line. (The value of $Z_0=90$ Ω is a result of our past experience with capacitance sensitivity.) To connect the resonator to the microfluidic electrodes, two 150 μm diameter brass wires are soldered to the printed circuit: one directly to the center transmission line and the other to the ground plane. The ends of the wires are cut to form sharp contacts. The resonator is mounted onto an x,y,z manipulator with 25 mm of travel for each axis and positioned to contact the microfluidic device under an optical microscope. Once the proper contact (both wire probes on the electrode pads) is established, the microwave driving frequency is adjusted to the resonant frequency of the resonator-microfluidic device system.

The brass wires, signal electrodes, and microelectrodes all increase the capacitive load on the printed resonator and thus the effective electrical length of the resonator. The resonance frequency is therefore reduced. This increases electrical losses and results in a decrease in the Q -factor [see Eq. (A9)], which drops from about 50 (without coupling to the microfluidic device) to about 10 (when the channel is filled with water).

IV. CIRCUIT MODEL

A simple circuit model was used to approximate the electrical behavior of the resonator-microfluidic sensor, where each component of the sensor is represented by a series of simple lumped elements. Lumped element values are based on a first order model of the physical component. The response of the sensor can be studied through simple modification of the circuit elements. For example, when fluid medium with different conductivity is introduced into the channel, the model for the microelectrode impedance can be modified by the addition of capacitance (to account for the change in dielectric constant), as well as the resistance (to account for the change in electrical loss).

FIG. 3. Circuit model for the resonator sensor when coupled to the microelectrodes in the channel. Input and output quarter-wavelength transmission lines 1 and 2 are edge-coupled to the center resonator C; symmetry allows them to be used interchangeably. All three lines are $l_r=25.7$ mm long; widths of lines 1 and 2 are $w_1=w_2=4.4$ mm, with the center line $w_c=1.0$ mm wide. Gaps between the lines, g , are both 4.2 mm. Center resonator C is effectively short-circuited on the left using an 82 pF capacitor. As indicated, low frequency signals (V_{DEP} in Fig. 1) can be applied to the electrodes through the 750 Ω resistor. The 0.27 nH inductance models via at the end of the resonator. The open-circuit end of the center resonator is coupled to a microfluidic array on the right using the brass wire probes. Conductance $G_{\text{rad}}=1/(40 \text{ k}\Omega)$ represents the effect of radiation losses from the resonator edges. The 0.1 pF capacitance models the brass wires; the 0.19 pF capacitor, 300 k Ω parallel resistor, and the 5.3 Ω series resistance model the wider electrodes. For a channel filled with DI water, microelectrodes are modeled by a 0.052 pF capacitor with 9.0 k Ω resistor. This corresponds to a sensor connected to a single gap electrode array, with losses expected for water at the 1.571 GHz operating frequency.

A. Description

Schematic representation of the sensor model is presented in Fig. 3. The electrical elements model the printed circuit and microfluidic device between points 1 and 2 in Fig. 1. The resonator is modeled using a coupled transmission line-distributed circuit, while the microfluidic device is modeled using lumped circuit elements—these include brass wires, interconnecting electrodes, and channel microelectrodes. As indicated in Fig. 3, a cell passing over the electrodes is included by a capacitance ΔC_{cell} ; cell capacitance can be modeled using Eq. (5) and numerical simulations of the field between the electrodes,²⁰ which depend on the cell position in the channel.

The circuit is simulated using ANSOFT DESIGNER microwave circuit simulator, with model parameter values initially obtained from physical dimensions and use of first order physical models. The capacitance due to the brass wires was determined experimentally. Measurements were performed with a network analyzer (Anritsu MS2036A).

B. Comparison of model to measurements

Model comparison and parameter extraction were conducted in three stages: (1) printed circuit resonator/brass wire probes not in contact with the microfluidic device interconnecting electrodes (in Fig. 3, this situation would correspond only to the resonator part of the circuit); (2) resonator/brass wire probes in contact with a microfluidic device, channel filled with de-ionized (DI) water, and sensor connected to a single gap microelectrode; and finally (3) resonator/brass wire probes in contact with a microfluidic device, channel filled with DI water and sensor connected to four-gap microelectrodes.

Stages (2) and (3) are modeled by the complete circuit in Fig. 3, with different circuit element values depending on which microelectrode array is connected. For DI water, we used $\epsilon_w=(\epsilon'_r-j\epsilon''_r)\epsilon_0$, with $\epsilon'_r=78$, and $\epsilon''_r=11$.^{28,29} [In Eq. (7), $\epsilon=\epsilon'$, with ϵ'' absorbed into “effective conductivity,” σ , i.e., $\epsilon'+\sigma/\omega\rightarrow\sigma$.) The conductivity of DI water was assumed to be $\sigma=10^{-3}$ S/m and therefore negligible at 1 GHz.

FIG. 4. Magnitude (top) and phase (bottom) of the power transfer function for (1) probes up, (2) water filled channel with single-gap microelectrodes, and (3) water filled channel with four-gap microelectrodes. Magnitude plot shows results of both measurements (solid) and simulations (dashed). Only measurement results are shown on the phase plot; intervals around resonant frequencies are highlighted in bold. Note how the slope decreases from (1) to (3) as the signal gets phase-shifted. In the phase plot (b), the path length phase dependence has been removed and only the phase response of the resonator element in the sensor is shown. Resonant frequencies are found at $f_{0,1}=1.850$ GHz, $f_{0,2}=1.566$ GHz, and $f_{0,3}=1.425$ GHz, respectively. The corresponding quality factors obtained through measurements are $Q_1=49$, $Q_2=26$, and $Q_3=10$. While the resonant frequencies obtained from simulations match the measured values very well, measured quality factors are, on average, about 25% lower than the values from the simulations. Measured data were obtained for a *finite* sized ground-plane resonator (GPR), while the simulated data were for an *infinite* GPR; this is possibly the reason for discrepancy between the two above the resonant frequencies.

The channel values specified in Fig. 3 (capacitance $C=52$ fF and resistance $R=9.0$ k Ω) are for case (2), single gap electrodes. The case of a four-gap microelectrode in stage (3) was modeled using the single gap values with $C=4 \times 52$ fF=210 fF and $R=9.0$ k $\Omega/4=2.25$ k Ω .

The simulated and measured transmission coefficients (rf_{out}/rf_{in}) as a function of frequency (“power transfer function”) for the sensor are given in Fig. 4 for each stage as described above. Solid lines represent results obtained by circuit simulation, while the dashed lines refer to those obtained by measurements. From the results, we determine the resonant frequencies $f_{0,i}$ for each stage and subsequently calculate the respective loaded quality factors Q_i using

$$Q_i = \frac{f_{0,i}}{B_i}, \quad (9)$$

where $f_{0,i}$ is the resonant frequency for the corresponding peak and B_i is the -3 dB of peak bandwidth. For case (3) and using Eq. (A13), with the characteristic impedance of the center resonator $Z_0=90$ Ω (a result of our past experience with capacitance sensitivity; see Ref. 19), we find that the sensitivity of the resonator is $|\Delta f/\Delta C| \approx 0.8$ MHz/fF. Similarly, from Eq. (A14) and with $Q=10$, $f_0=1.45$ GHz, we estimate that $|\Delta \phi/\Delta C| \approx 2.57 \times 10^{-3}$ rad/fF = $4.49 \times 10^{-5}^\circ$ /fF.

Resonant frequencies are found at $f_{0,1}=1.843$ GHz, $f_{0,2}=1.571$ GHz, and $f_{0,3}=1.435$ GHz using simulations. Measured counterparts are $f_{0,1}=1.850$ GHz, $f_{0,2}=1.566$ GHz, and $f_{0,3}=1.425$ GHz. Increased capacitive and resistive loadings decrease the resonant frequency, as can be seen from Fig. 4, and cause the quality factor Q [cf. Eq. (A9)] to decrease. From Eq. (9), quality factors obtained through measurement are $Q_1=49$, $Q_2=26$, and $Q_3=10$. Resonant frequencies obtained from simulations match the measured values very well; however, peaks are wider in measured cases, and Q factors appear to be on the average 25% lower than their simulated values. Although we accounted for damping effects in simulations, there may be additional losses that we did not consider; nevertheless, the strongest contribution to the above discrepancy in quality factors is likely from the use of simple first order physical model for lumped circuit elements values.

V. SYSTEM CALIBRATION

Calibration of the sensitivity of our device is performed using a sample with well defined properties. We have used 5.7 ± 0.3 μm diameter polystyrene spheres (PSSs) from Polysciences, Inc. The size of the spheres is well defined and their dielectric constant and conductivity are $\epsilon = 2.5\epsilon_0$ and $\sigma = 10^{-4}$ S/m, respectively.

Computer simulation of a single polystyrene bead passing at different heights above the electrodes provides the expected ΔC and thus the expected signal profile [using Eq. (8)]. This can be compared with measured data obtained using the PSS sample.

A. Simulations

As outlined in the Sec. II, we calculate the change in capacitance caused by the passage of particle through a given region near a microelectrode array using Eq. (5). To this end, COMSOL® MULTIPHYSICS is employed. The nonuniform electric field produced by the coplanar electrodes is obtained by solving the Laplace equation $\nabla \cdot (\epsilon \nabla \phi) = 0$ (where ϕ is the potential of the field at any given point) with appropriate boundary conditions and using $\mathbf{E} = -\nabla \phi$. The channel is modeled with a uniform rectangular cross section and a laminar Poiseuille flow is assumed.³⁰ The change in capacitance, ΔC , is determined numerically as the difference in capacitances as calculated before and after the particle is inserted into the channel. This change is proportional to $|\mathbf{E}_{\text{rms}}|^2$ [see Eq. (5)], and the sign of ΔC is determined by the sign of K_{CM} , which, in turn, depends on whether the particle polarizes more or less than the surrounding medium. In all examples and applications presented in this paper, ΔC is negative.

In simulations, the particles are modeled as uniform spheres of dielectric permittivity ϵ_p . This is always appropriate for the PSSs. For yeast and Chinese hamster ovary (CHO) cells, however, simulated ϵ_p corresponds to an “effective” value of dielectric permittivity for a given cell. The effective permittivity for a yeast cell, for example, is determined using a double-shell structure model.^{20,26}

The signals resulting from particles flowing at different distances above the electrodes will look similar to profiles shown in Fig. 5. Simulations in Fig. 5 are for an individual bead passing over five interdigitated electrodes in a channel 25 μm deep. Electrodes are 25 μm wide with a 25 μm gap. We assume that the particle stays within the same fluid layer as it passes over the electrodes (i.e., constant height and speed). Simulation was repeated for different heights above the electrodes (5–10 μm in steps of 1 μm as well as 12.5, 15, and 20 μm). For large heights, the electric field appears strongest in the middle of the gaps (rounded peaks start to appear at about 10 μm). The profile becomes more detailed at lower heights, where the peaks split, indicating regions of strong field around the edges of the electrodes. As can be clearly seen from Fig. 5, there is a significant difference between the profiles, particularly near the bottom third of the channel. We have used this to determine the position of a particle within a channel to within a nearest micrometer resolution.

FIG. 5. Simulation of $|E_{\text{rms}}|^2$ and the related capacitance change for different PSS heights above the electrodes. On the left, successive contours correspond to steps in distance from the channel floor. (Note that the steps are not equal beyond 10 μm mark.) Above the plot is a cartoon of the channel with a 5.7 μm PS bead passing over the electrodes. The field is strongest around the electrode edges and least strong around the middle points of the electrodes. As the distance from the floor increases, contours become more closely spaced and begin to lose their distinct profiles. For example, above a certain height, it is not possible to infer the location of the electrode edges (compare the contours for 10 and 12.5 μm). On the right, peak-to-peak capacitance (proportional to $|E_{\text{rms}}|^2$) is shown. The region of dramatic change (5–10 μm) allows us to determine with confidence the distance at which the bead is passing above the electrodes (see Fig. 6 and Sec. VI A for more details).

B. PSSs calibration

To calibrate the sensor system, we experimentally measure the signals produced when PSS flow above the electrodes in the microfluidic channel. We then compare these signal profiles to the profiles obtained via COMSOL[®] simulation. Matching profiles allows us to estimate—within 1 μm —the distance above the electrodes at which the spheres have passed by. In the typical example provided in Fig. 6, the signal profile is best matched by a bead flowing at 6 μm above the bottom of the channel (see figure caption for explanation). To estimate the signal strength, we use a point between the peaks corresponding to the instant the bead is passing above the midgap between the second and the third electrodes as a reference. This point is the least sensitive to changes in height. The signal strength at this point, $S=2$ V, corresponds to ΔC of approximately 50 aF.

To estimate the capacitance sensitivity, we measure the root-mean-square (rms) noise δS (in the example in Fig. 6 this was done using a 2 s interval). Since the change in capacitance, ΔC , is directly proportional to the strength of the signal S [Eq. (8)], we can specify

$$\delta(\Delta C) = \frac{\delta S}{S} \Delta C. \quad (10)$$

This typically results in zeptofarad sensitivity. For example, using data from the result in Fig. 6, we obtain $\delta S=0.026$ V from the interval 0–2 s. This results in $\delta(\Delta C)=650$ zF for a LIA time constant of 3 ms.

VI. TYPICAL EXAMPLES AND APPLICATIONS

We provide three illustrative examples to demonstrate the application of the detection system described above. Our measurement samples include commonly used and well-characterized materials, microorganisms and cells such as PS microspheres, baker's yeast, and CHO cells.

FIG. 6. Measured signal for a PSS passing above the electrodes is shown on the left. On the right, simulation results from Fig. 5 are shown as individual contours. We compare the midpath region (shown in bold) of the measured signal (in the channel, this region would correspond to the distance from the center of the second to the center of the third electrode). The relative magnitude and the shape of the signal appear most consistent with a sphere passing at a distance of 6 μm above the electrodes.

A. Polystyrene microspheres velocity versus distance

Our first illustration is the stationary flow of PSSs suspended in DI water. We measured the peak-to-peak amplitude and full-width half-maximum (FWHM) of the signal [see inset of Fig. 7(a)] as PSSs passed over a two-gap interdigitated electrodes. Simultaneous video recordings confirmed that the amplitudes corresponded to the passage of single particles. Assuming a Poiseuille flow³⁰ with a parabolic velocity profile with a maximum velocity of approximately 1300 $\mu\text{m/s}$, and using the results of our simulations, we obtain a model prediction for the relationship between the signal amplitude and transit time over the electrodes. The resulting model curve, along with experimental data (97 spheres in total), is shown in Fig. 7(a). Since the strongest signal correlates with slower particles passing just above the electrodes and vice versa, the upper branch of the model corresponds to the lower half of the channel. The distance of nearest approach above the electrodes and below the channel top—about 3 μm for the 5.7 μm diameter spheres—determines the end points on the model curve. Data were fitted to the model using a reference point at 180 ms FWHM.

In the time required to achieve stationary flow, the suspension of PSSs settles under the influence of gravity, and most particles are detected below the middle of the channel. (Hydrodynamic lift forces³¹ prevent particles from settling all the way to the channel floor.) The dependence of the signal amplitude on the transit time is almost linear, allowing us to determine the velocity of the particle and height above the electrodes with reasonable accuracy (cf. Ref. 32). Histograms of signal amplitudes and bead velocities [Fig. 7(b)] reveal dominant-peaked distributions of position and velocities of the particles within the channel: 86% of the beads passes above the electrodes at 6 μm or lower; 72% of the beads had velocities within the narrow interval of 400–600 $\mu\text{m/s}$. The velocity histogram agrees well with the predicted flow rate and demonstrates that particle velocities are determined by the velocity profile of the layers of suspending fluid.

B. Yeast cells in salt solution

Yeast is the most thoroughly researched eukaryotic micro-organism. As an often used model cell in microbiological studies, it was an obvious choice for study with our system. Baker's yeast

FIG. 7. (a) Model prediction and measured data for 97 PSSs with $5.7 \mu\text{m}$ diameter. Inset shows how amplitude and FWHM were determined for each sample signal. In addition to the four peaks corresponding to the electrode-gap edges two small peaks appear on either side of the signal, associated with outer edges of the first and the last electrode. (b) Altitude and velocity histograms show dominant-peaked distributions of positions in the channel and velocities.

(*Saccharomyces cerevisiae*) was suspended in a 90 ppm NaCl solution and pumped through the microfluidic channel.

Figure 8 shows characteristic signals produced as yeast particles flow over the three-electrode array (two gaps)—as shown in the inset micrograph. During the measurement interval, three particles have passed by. The signal-to-noise ratio is very good and the signal is clearly resolved. The time taken to cross the electrodes can be used to measure velocity and may have application in field-flow fractionation measurements.^{31,33} For the first two signals, note that the third peak is slightly smaller than the second one, indicating that the yeast cell is gently pushed away from the electrodes due to small dielectrophoretic (DEP) forces for $\epsilon_p < \epsilon_m$.

C. CHOs in tris and phosphate buffer saline buffer

As an example of a mammalian cell we have chosen the CHO cell. These cells are widely used in pharmacological applications for expression and large-scale recombinant protein production. Mammalian cells often require buffer solutions with high salt concentrations; we suspended CHO cells in a phosphate buffer saline (PBS) solution.

FIG. 8. Typical signal for yeast cells. Peaks occur near electrode edges. Note the two smaller peaks at the beginning and end of the signal, associated with the outer edges of the first and the last electrodes.

The signal produced by a CHO cell is shown in Fig. 9. The signal is greater in amplitude than either the yeast or PSS signal. This is to be expected considering the size of these cells: 15–18 μm diameter on the average, compared with approximately 6 μm diameter for PSSs and 6–8 μm diameter for yeast. The distance to the electrodes is determined from the physical center of the cell, which is higher up above the electrodes than it would be for a PS bead or a yeast cell. Consequently, we expect the signal profile to be more consistent with the larger height simulation

FIG. 9. Typical signal for a CHO cell.

contours (compare with 10 μm contour in Fig. 5). Note that in addition to being less pronounced (i.e., having rounded rather than “split” peaks), this signal lacks two “extra” peaks associated with outer electrode edges [compare with signals for PSSs and yeast in respective Figs. 7(a) and 8].

Thorough measurements of dielectric permittivity across a wide range of frequencies were not yet performed for CHO cells; in the absence of data, one can use estimates from other cells, particularly at gigahertz frequencies. A typical mammalian cell has ϵ between $40\epsilon_0$ and $70\epsilon_0$ (see, e.g., Ref. 22); the permittivity of CHO cells was recently measured to be $60.2\text{--}62.9\epsilon_0$ within the frequency region of 1.7–2.6 GHz.³⁴ Comparing the amplitude of the signal for CHO cells in two different suspending media of known dielectric constant and conductivity (e.g., PBS and Tris), would, in principle, yield detailed information on the Clausius–Mossotti factor from which we could extract the average value of ϵ_{CHO} , thereby providing electronic characterization of the cell.

VII. SUMMARY AND CONCLUSIONS

We have developed a sensor with zeptofarad sensitivity operating within an all-electronic device in the gigahertz range of frequencies. High sensitivity detection makes it possible to sense individual bioparticles and biomaterials at very low concentration or perhaps even identify cells at different stages of their development and provide their electronic characterization. We presented an overview of the components of our apparatus, discussed how to calibrate the system, and provided an appropriate circuit model for the sensor. Through a display of different results we demonstrated the ability to simultaneously evaluate velocity and position of a particle within the channel and subsequently infer the trajectory of the passing cell. The circuit model that we developed can be adapted to suit modifications of the system (e.g., different dielectric suspensions), which could then be easily tested.

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APPENDIX: RATE OF CHANGE OF PHASE SHIFT WITH CAPACITANCE

To obtain the change in phase as a function of load capacitance, $(\partial\varphi/\partial C)|_{f_0}$, for the coupled line resonator, we employ

$$\left. \frac{\partial\varphi}{\partial C} \right|_{f_0} = \left. \frac{\partial\varphi}{\partial f} \right|_{f_0} \left. \frac{\partial f}{\partial C} \right|_{f_0} \quad (\text{A1})$$

and then evaluate the individual partial derivatives. To calculate the term $\partial\varphi/\partial f$, we model our resonator as a low-loss transmission line of length l and terminated in a short-circuit (i.e., zero load impedance, $Z_L=0$) low-loss transmission line.³⁵ For this case, the input impedance can be written as

$$Z_{\text{in}} = Z_0 \tanh(\alpha + j\beta)l, \quad (\text{A2})$$

where Z_0 is the characteristic impedance, α is the attenuation constant, $\beta=2\pi f/v=2\pi/\lambda$ (with v representing the phase velocity), and $j=\sqrt{-1}$. We can rewrite Eq. (A2) as

$$Z_{\text{in}} = Z_0 \frac{1 - j \tanh \alpha l \cot \beta l}{\tanh \alpha l - j \cot \beta l}. \quad (\text{A3})$$

For a quarter-wavelength resonator, with $l=\lambda_0/4$ at the resonant frequency f_0 , $\beta l=\pi/2$. Close to the resonant frequency, $f=f_0+\Delta f$ and

$$\beta l \approx \frac{\pi}{2} + \frac{\pi \Delta f_s}{2 f_0}. \quad (\text{A4})$$

For a low-loss line $\alpha l \ll 1$, and Eq. (A3) can be approximated as

$$Z_{\text{in}} = Z_0 \left(\alpha l - j \frac{\pi \Delta f_s}{2 f_0} \right)^{-1}, \quad (\text{A5})$$

with the phase $\Delta \varphi$ near resonance given by

$$\tan \Delta \varphi = \frac{\Im\{Z_{\text{in}}\}}{\Re\{Z_{\text{in}}\}} = \frac{\pi \Delta f_s}{2 \alpha l f_0}. \quad (\text{A6})$$

For $\Delta \varphi$ sufficiently small, $\tan \Delta \varphi \approx \Delta \varphi$. Thus, for $\Delta f_s \rightarrow 0$,

$$\left. \frac{\partial \varphi}{\partial f} \right|_{f_0} = \frac{\pi}{2 \alpha l f_0}. \quad (\text{A7})$$

The attenuation constant α can be expressed as³⁵

$$\alpha = \frac{\pi}{Q l}, \quad (\text{A8})$$

where Q is the quality factor, defined for a low-loss resonator as

$$Q = \frac{f_0}{B}, \quad (\text{A9})$$

with B the -3 dB peak bandwidth (i.e., the width where the power is half of its peak value). Thus, the first term in Eq. (A1) is

$$\left. \frac{\partial \varphi}{\partial f} \right|_{f_0} = \frac{Q}{2 f_0}. \quad (\text{A10})$$

To find the rate of change of frequency with load capacitance $\partial f / \partial C|_{f_0}$, we consider the quarter-wavelength transmission line of the length $l = l_0 = \lambda_0 / 4 = v / 4 f_0$ at the resonant frequency f_0 . For a capacitance per unit length C' , an inductance per unit length L' , the characteristic impedance is $Z_0 = \sqrt{L' / C'}$ and the phase velocity is $v = 1 / \sqrt{L' C'}$.

Once the sensor is coupled to the electrodes, the load capacitance C causes the effective length of the resonator to change to $l_0 + \Delta l$, forcing the resonant frequency to shift to

$$f = \frac{v}{\lambda_s} = \frac{c}{4(l_0 + \Delta l)}. \quad (\text{A11})$$

For small Δl , the resonant frequency can be related to the load capacitance as

$$f \approx f_0 \left(1 - \frac{4 f_0}{v} \Delta l \right) = f_0 (1 - 4 f_0^2 Z_0 C' \Delta l). \quad (\text{A12})$$

We can express the capacitive load as $C = C' \Delta l$. Thus, for small Δl (i.e., for small C), we can evaluate $\partial f / \partial C|_{f_0}$ as

$$\left. \frac{\partial f}{\partial C} \right|_{f_0} = -4 f_0^2 Z_0. \quad (\text{A13})$$

Finally, Eq. (A1) can be evaluated by substituting the results of Eqs. (A10) and (A13) to obtain

$$\left. \frac{\partial \varphi}{\partial C} \right|_{f_0} = -2Qf_0Z_0. \quad (\text{A14})$$

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