Electrical characterization of DNA molecules in solution using impedance measurements

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We report on impedance measurements of fluids to examine the impact of the length and concentration of free-floating double-stranded DNA molecules. The impedance versus frequency characteristics were fitted to an equivalent circuit model including solution dielectric capacitance and conductance. The extraction of these parameters can be used to detect the presence of DNA molecules in the nanomolar range for a 400 bp long molecule. Our results show that the extracted dielectric capacitance and conductance increase with molecule length and concentration owing to a corresponding increase in number of molecule dipoles and counterions in solution. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2908203]

Label-free detection of DNA molecules is a quest of utmost importance. The direct electrical detection of DNA in solution is potentially a very attractive option, especially if differences in length and concentrations can be directly detected without any labels or attaching the molecules to a surface. The basic mechanism behind the electrical response of DNA molecules in solution under an applied alternating electrical field stems from the formation and relaxation of the induced dipole moment and these investigations date back to Jungner *et al.* in 1949.[1] Since then, electrical properties of DNA in aqueous solutions have been explored and the studies clearly prove that since there is mobile charge in and around the DNA, a dipole can be induced in the DNA when electrically probed in solution.[2,3] To-date, most of the label-free DNA detection mechanisms utilize the DNA attached to surface electrodes.[4–6] However, there are a few reports of electrical measurements to detect DNA molecules in solution, with sizes ranging from 20-mer oligonucleotides to λ-DNA.[7,8] The presence of λ-DNA at concentration of 10 ng/μl was detected in 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA) by comparing the measured impedance and capacitance to that of de-ionized water or TE buffer without the DNA.[7] In addition, 20-mer ssDNA oligonucleotides at concentration of 100 nM could be detected in fluid by a nanogap device with gap size of 20 nm.[8] These reports show the promise of label-free DNA detection in solution; however, more studies are needed to explore the sensitivity and limits of detection and the physics behind the detection mechanism. Electrical characterizations of DNA of different lengths and concentrations in solution have been reported,[9,10] and the results showed correlation between changes in solution impedance and the DNA molecules. The measured impedance in all these previous studies was an aggregate of electrical behavior of all the ions and molecules in solution. We take these studies to the next step by showing the explicit impact of DNA molecules on the solution capacitance and resistance. We show that the solution with DNA can be modeled as a lump electrical circuit and that the changes in impedance of the solution with the DNA can be correlated to

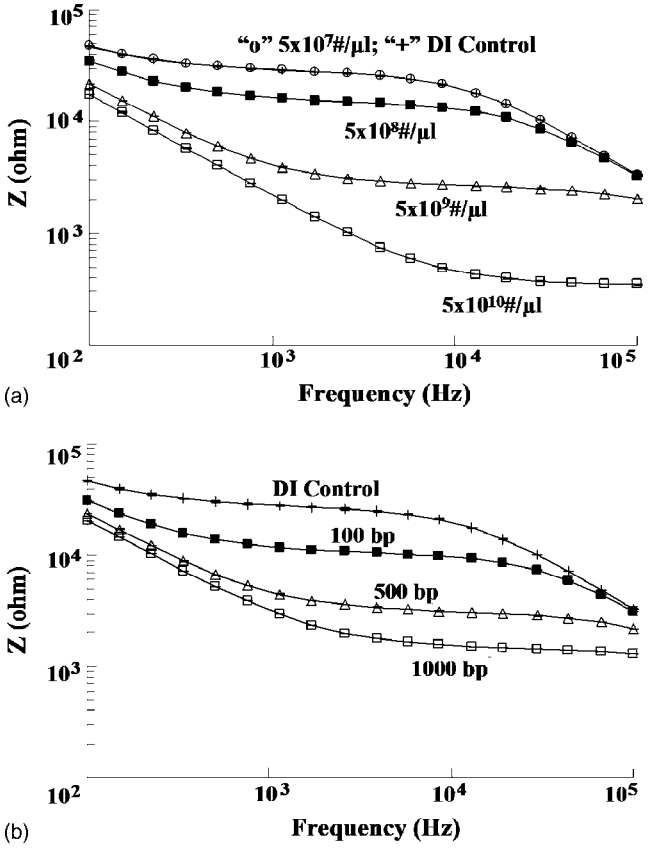

FIG. 1. Impedance magnitude as a function of frequency for (a) variation in concentration of 400 bp dsDNA molecule, and (b) variation in size of dsDNA where concentration of each molecule was 10^9 molecules/μl.

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the concentration of DNA. Our measurements also reveal further insight into the dipole moment of the DNA molecules as a function of their length.

The detection electrodes of our device was fabricated by metal evaporation of 50 nm Ti and 250 nm of Au, 25 μm wide and 25 μm spacing, onto a 3500 ± 200 Å thermal SiO_2 layer grown on a silicon substrate. A (poly)dimethylsiloxane (PDMS) well was made from 10:1 mixture of elastomer base:curing agent and cured in a hard-bake oven at 120 °C for 10 min. The DNA molecules were prepared by gel extraction and ethanol precipitation. The DNA molecules were always resuspended in de-ionized water for all the measurements described here. The purity of the DNA samples was confirmed by a spectrophotometer, i.e., A260/A280 ranges between 1.8 and 2.0 (Nanodrop, Wilmington, DE). 10 μl volume of the prepared DNA solution was pipette into the PDMS well with measurement electrodes underneath. An ac voltage with amplitude of 250 mV was applied to the electrodes and impedance was measured with frequency varying from 100 Hz to 1 MHz. Each scan with 19 steps took about 1 min to complete and three sweeps were taken for each experiment and averaged.

We first measured the impedance of the DNA solutions as a function of concentration and length of the DNA molecules, as shown in Figs. 1(a) and 1(b). The impedance magnitude was found to decrease as the concentration of the 400 bp dsDNA was increased. The detection limit was found to be around 1 nM for the 400 bp dsDNA molecule. Similarly, at the concentration of 10^9 molecules/μl, the impedance magnitude was also found to decrease as the length of the dsDNA molecule was increased. Experiments were also performed to further investigate the dependence of impedance change as a function of the total number of base pairs of the dsDNA in solution. Three different sizes, i.e. 50, 500, and 5000 bp samples were used, each with a different concentration such that the total number of base pairs in the solution was kept the same. As shown in Fig. 2, we observed the same general trend in changes in impedance due to DNA concentrations, i.e., impedance decreases as the concentra-

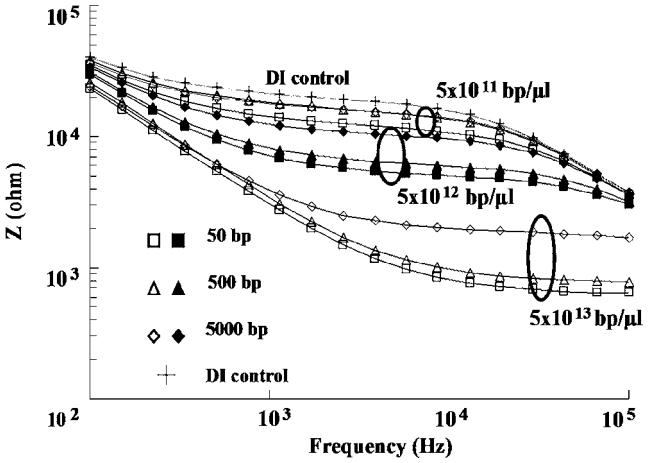
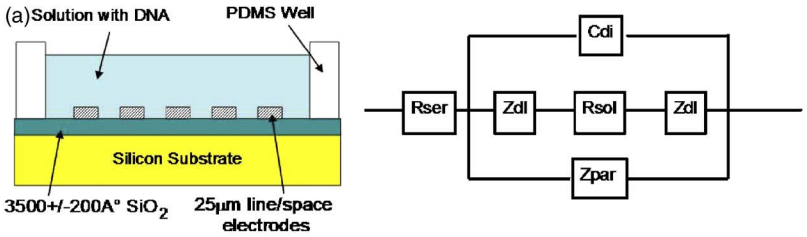
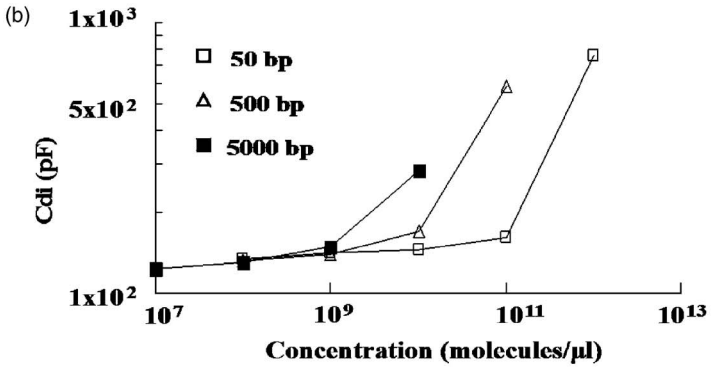


FIG. 2. Impedance versus frequency for the different size ethanol-precipitated dsDNA molecules.





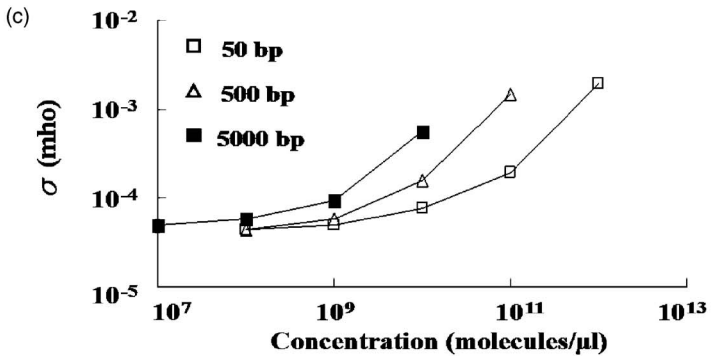


FIG. 3. (Color online) (a) Left: drawn schematic of the device used for the measurements. Right: equivalent circuit model of the solution with DNA molecules. (b) Extracted solution dielectric capacitance as a function of molecule concentration. (c) Extracted solution conductance as a function of molecule concentration.

TABLE I. Extracted circuit model parameters for varying concentration and size of DNA molecules.

	R_{sol} (kΩ)	C_{di} (nF)	n	$B(\times 1e-7)$
Concentration of 400 bp DNA Molecules				
$5e7$ molecules/μl	31.2	0.174	0.910	1.73
$5e8$ molecules/μl	16.3	0.191	0.896	2.04
$5e9$ molecules/μl	2.64	0.311	0.897	2.85
$5e10$ molecules/μl	0.21	2.020	0.905	3.30
Length of DNA Molecules at concentration of $1e9$ #/μl				
100 bp	11.2	0.206	0.893	2.22
500 bp	3.04	0.292	0.890	2.72
1000 bp	1.32	0.400	0.888	3.14

tion increases. Interestingly, when the curves were grouped for the different size DNA with same total amount of base pairs, as shown in Fig. 2, an interesting trend emerges. For the cases of 50 and 500 bp samples, same number of base pairs (molecule length × concentration) showed similar solution impedance, whereas for the 5000 bp samples, the impedance was higher.

To further examine the physics underlying this observed phenomena, we fitted the measured data of interdigitated electrodes structures and electrolyte[11,12] (Fig. 3, left) to an equivalent circuit model (Fig. 3, right). The device parasitic impedance originating from the oxide capacitance and the substrate resistance is defined as $Z_{par}=[(j2\pi f)^n B]^{-1}$. R_{ser} is the fixed resistance of the metal lines leading to the interdigitated electrodes. Both R_{ser} and Z_{par} were extracted from impedance measurement of the device without electrolyte and kept fixed for the rest of the analysis. The extraction of the model parameters was performed using MATLAB (The MathWorks, Natick, MA) by iterating each parameter and minimizing the least square error between the model and the experimental data.

When the data from the first experiment was analyzed, as shown in Table I, the extracted electrical double layer impedance shows only a small perturbation from experiments to experiments proving that the dominant changes are taking place in the solution itself and not at the interfaces. The solution dielectric capacitance is defined as $C_{di}=\kappa\varepsilon_{sol}\varepsilon_o/K$, where K is a geometrical factor of the electrode structures. The $\kappa\varepsilon_{sol}$ is the relative dielectric permittivity of the solution and is explicitly affected by DNA dipole moment (DNA length × charge) and concentration of the DNA. The changes in the extracted dielectric capacitance (C_{di}), which represents the DNA capacitance due to the movement of the counterion charges around the DNA backbone (dielectric relaxation of DNA dipoles), was also found to vary with changes in size and concentration of DNA molecules in solution. The model extraction results from the measured data are presented in Figs. 3(b) and 3(c). It can be noted from Fig. 3(b) that the values of C_{di} for samples with 50 bp at 10^{10} molecules/μl, 500 bp at 10^9 molecules/μl, and 5000 bp at 10^8 molecules/μl (which all have the same number of total base pairs) are close to the same detection limit. For increasing concentrations of any of the molecule lengths in Fig. 3(b), the extracted value of C_{di} increases as expected, since an increase in concentration should result in an increase in the number of the dipole moments. This should also hold for an increase in molecule length at a fixed concentration (dipole moment=DNA length × charge). However, it should be noted that dsDNA molecules with lengths greater than the persistence length of about 50 nm (~130 base pairs)[13] would start to bend and longer molecules would coil, especially at low ionic strengths. Hence, the effective separation between the opposite ends of the dipole charges will not directly scale with the length of the molecule for longer molecules. As shown in Fig. 3(c), the trend was also similar for the extracted bulk solution conductance ($\sigma=1/R_{sol}$), which was found to increase as both the DNA concentration and length were increased. This can be explained by the fact that an increase in DNA concentration could result in a higher number of counter-ions attracted by the backbone charge, as the molecules are precipitated and concentrated from the original source. The increased number of mobile counter-ions can increase the conductivity of the solution. This is consistent with studies of single DNA molecules translocating through nanopore channels in low ionic conductivity solutions which found that the ionic currents can be enhanced due the mobile counter-ions around the DNA.[14,15]

In summary, we present detailed characterization of label-free electrical detection of DNA using impedance measurement in fluid. The results show that the impedance magnitude decreases as the concentration or the length of the DNA is increased and the detection limit for a 400 bp long molecule in de-ionized water was found to be in the nanomolar range. Based on extraction of circuit model parameters, it was demonstrated that the changes due to concentration and sizes of DNA are directly related to the total number of base-pairs in the solution and the conductance and the capacitance of the solution increases with DNA concentration and length. The detections of differences in the length of the free-floating DNA molecules with fixed concentration and differences in concentration with fixed DNA length can potentially be used as a way to provide a label free method to detect DNA products in solution.

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