Dielectrophoresis of DNA: Quantification by impedance measurements

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Dielectrophoretic properties of DNA have been determined by measuring capacitance changes between planar microelectrodes. DNA sizes ranged from 100 bp to 48 kbp, DNA concentrations from below 0.1 to 70 $\mu\text{g/ml}$. Dielectrophoretic spectra exhibited maximum response around 3 kHz and 3 MHz. The strongest response was found for very long DNA (above 10 kbp) and for short 100 bp fragments, which corresponds to the persistence length of DNA. The method allows for an uncomplicated, automatic acquisition of the dielectrophoretic properties of submicroscopical objects without the need for labeling protocols or optical accessibility. © 2010 American Institute of Physics. [doi:10.1063/1.3430550]

I. INTRODUCTION

In micro- and nanofluidics, alternating electric (ac) fields have found a steadily spreading range of applications for the spatial manipulation of micro- and nanoparticles, biomacromolecules, and fluids. Quite diverse phenomena have been observed and exploited: Electrostretching of soft material such as mammalian cells¹ and of filamentous molecules such as DNA,^{2,3} electro-orientation of elongated objects such as fission yeast⁴ and electrostretched DNA,⁵ electrorotation of cells,⁶ and electropumping of fluids^{7,8} and ac electro-osmotic flow.⁹ Still, the most widely exploited effect is dielectrophoresis (DEP),^{10–12} i.e., the action of forces in inhomogeneous electric fields toward regions of highest field strength (positive DEP) or lowest field strength (negative DEP). Like the other ac electrokinetic phenomena, it varies with physical parameters, e.g., conductivity and polarizability of media and particles, and with electronic parameters such as frequency, phase, and amplitude of the applied field. Often the term DEP is used in a somewhat wider sense when phenomena, such as electrostretching, electro-orientation, and DEP, take place simultaneously, as it is often the case when dealing with long DNA strands.^{2,5} Sometimes it is not obvious whether an observed accumulation of particles is caused by DEP or ac electro-osmosis. Although DEP of microscopical particles such as cells has been well characterized by both experiment and theory,^{2,10,13–16} there are not many quantitative studies on submicroscopical objects, and even less on molecules.¹² For latex spheres and viruses, the crossover frequency, i.e., the frequency at which positive DEP changes to negative DEP, has been determined systematically by fluorescence microscopy.^{17,18} Washizu *et al.*¹⁹ measured the dependence of DEP of fluorescently labeled DNA on field intensity by DEP chromatography. Fluorescence microscopy with image analysis has been used to determine DEP response of DNA by monitoring fluorescence intensity near the electrodes^{20–24} or at isolating constrictions.²⁵ All these methods require the object to fluoresce, in most cases to be fluorescently labeled. This makes sample preparation more laborious and may introduce artifacts due to interaction of the dye both with the molecule of interest and with the electrical field. Above this, photobleaching of the dye leads to a decrease in fluorescence with time and photocission may even disintegrate the DNA strand.²⁶ As a label-free alternative DNA concentration can be monitored by impedance spectroscopy or capacitance measurements, respectively,^{27,28} using the same electrodes for manipulation and for sensing. This combination

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was first applied by Milner *et al.*²⁹ on bacteria. They used a differential electrode set and measured phase changes of a 500 Hz probing signal with a lock-in amplifier. The DEP signal of a second signal source was varied from 1 kHz to 10 MHz. Interference with the impedance measurement was avoided by passive filtering. Suehiro *et al.*³⁰ worked at a fixed frequency of 100 kHz, which served for both DEP action and impedance sensing. Signal analysis was done with an oscilloscope. Arnold³¹ monitored DEP response of yeasts at fixed frequencies ranging from 1 to 10 MHz through an impedance analyzer working between 50 kHz and 5 MHz. The sensing signal was separated from DEP excitation by a balanced transformer achieving a 200-fold suppression. DEP and impedance measurements on DNA samples were first studied by Hölzel and Bier²⁸ through a component analyzer. The analyzer served for both DEP excitation and measurement. Different excitations and sensing signals were employed by temporal decoupling, which means that DEP action and impedance sensing worked intermittently. Here it was exploited that redistribution of molecules after dielectrophoretic accumulation occurred on a much longer time scale than switching between DEP and sensing mode. This principle was later employed in a more sensitive system with a capacitance bridge, an independent signal source, and with switching by relays.³² In the present work this enhanced system is used for studying the influence of DEP frequency, DNA concentration, and molecular weight on the dielectrophoretic response of DNA.

II. MATERIALS AND METHODS

A. DNA preparation

Double stranded DNA fragments with 100 and 500 bp lengths were obtained from Fermentas (NoLimits DNA fragment, St. Leon-Rot, Germany) dissolved in buffer. They were dialyzed against ultrapure water to remove salts and to decrease electrical conductivity (Slide-A-Lyser, Pierce, Bonn, Germany). As a much longer sample M13 mp18 phagemid DNA (7231 bp, contour length of 2.4 μm) was prepared by transformation of *E. coli* cells (strain JM109, New England Biolabs, Frankfurt/Main, Germany) with M13 mp13RFI DNA (New England BioLabs). DNA was isolated through a QIAfilter Plasmid Midi-Kit (Qiagen, Hilden, Germany) and single stranded DNA was removed by digestion with Mung Bean Nuclease (New England BioLabs, Frankfurt/Main, Germany). Double stranded DNA was linearized by the restriction endonuclease EcoRI and contaminations were removed by washing using centrifugal filters (Microcon, Millipore, Schwalbach, Germany). λ -DNA (48 502 bp, 16 μm) from Fermentas was either dissolved directly in ultrapure water or it was cut into two 24 kbp fragments by the restriction enzyme XbaI (Fermentas) and then purified by repeated centrifugation and washing with ultrapure water in centrifugal filters (Microcon). These preparations led to stock solutions of around 70 $\mu\text{g}/\text{ml}$ concentration, which were further diluted by ultrapure water. DNA concentrations were determined by a spectrophotometer (Nanodrop ND-1000, Thermo Scientific, Schwerte, Germany).

B. Electrodes

Interdigitated electrodes were prepared from commercially available surface acoustic wave (SAW) resonators PARS 433.92 (Kyocera, Fountain Inn, SC). Their resonance frequency of 433.92 MHz is well beyond the maximum frequency of this work (3 MHz) and, hence, does not interfere with the experiments. These resonators consist of a quartz substrate with about 3 mm length and 0.7 mm width placed on a ceramic support, which is sealed by a metal top (Fig. 1). The interdigitated aluminum electrodes on the quartz are composed of 628 fingers with 150 μm length and 1.4 μm width each, separated by 1.1 μm gaps. They are surrounded by additional, electrically isolated electrodes that are not used in the present application. To make the electrodes accessible, the package's metal lid was removed mechanically. Electrodes were then rinsed with ultrapure water and inspected for contaminations and defects.

C. Experimental setup

The experimental setup was based on that described by Henning *et al.*³² (Fig. 2). For dielectrophoretic action the signal of a rf synthesizer (Hameg HM 8133-2, Kaiserslautern, Germany)

FIG. 1. Microelectrode chamber. A commercial SAW resonator with interdigitated microelectrodes on a quartz carrier. The top lid has been removed. $2\ \mu\text{l}$ samples were applied onto the electrodes and sealed with a cover glass.

was amplified by a power amplifier (Toellner TOE 7606, Herdecke, Germany) and fed to the electrodes through reed relays (Meder, Singen, Germany). Impedance measurements were performed by an ultraprecision capacitance bridge (Andeen-Hagerling AH 2550A, Cleveland, OH), which was also connected to the electrodes through relays. Both synthesizer and bridge were controlled by purpose-built software running on a personal computer. Relays were activated using the integrated switch panel of the capacitance bridge. Data acquisition was also computerized. The bridge worked at 1 kHz and voltages were limited to maximally $30\ \text{mV}_{\text{rms}}$ to minimize the impact on DNA distribution. DEP signals were varied stepwise between 1 kHz and 3 MHz at $3\ \text{V}_{\text{rms}}$. DEP frequency and amplitude were checked by a counter and a voltmeter. For each experiment

FIG. 2. Experimental setup. DEP signal source and capacitance bridge are alternately connected to the electrodes. Switching is performed by relays that are controlled via the bridge's integral switch panel. The DEP signal is generated by a rf synthesizer and a power amplifier. Instrument control and data acquisition are performed by purpose-built software.

FIG. 3. (a) Temporal course of capacitance during acquisition of a DEP spectrum starting at 3 MHz. The graph starts at $t=1000$ s since it represents the second of three consecutive spectra lasting 1000 s each. (b) Enlarged view for $t=1600, \dots, 1650$ s. ΔC is calculated from the capacitance change during the DEP period. (c) From the temporal course $(d/dt)C=\Delta C/\Delta t$ is calculated and averaged for each frequency.

2 μl sample was placed onto freshly prepared electrodes and covered with a cleaned microscope slide. Evaporation was prevented by a sealing oil film between SAW metal case and cover glass. As a consequence of the sample drop being smaller than the metal case's volume, it only came into contact with the quartz substrate, the aluminum electrodes, and the cover glass. This helped to avoid contaminations that would change the sample's electrical conductivity. Direct observation of DNA field response was done by fluorescence microscopy (Olympus BH-2, Hamburg, Germany) of 24 kbp fragments stained with PicoGreen (Invitrogen, Darmstadt, Germany). Fluorescence micrographs were acquired by a cooled charge coupled device camera (Kappa PS 2C) and gray values were determined for a fixed rectangular region in a central electrode gap by image analysis software (IMAGEJ).

III. RESULTS AND DISCUSSION

As a measure of DNA concentration and DEP response, only capacitance data have been taken into account. Although resistance changes were also registered, they were less pronounced and, therefore, have not been evaluated. After each DEP, field application lasting 8 s capacitance data were acquired for 2 s. This was repeated 11 times for each DEP frequency resulting in a duration of 960 s for a complete spectrum. In Fig. 3(a) the typical temporal course of the capacitance is shown. Overall capacitance increased with time, which is interpreted as a consequence of dielectrophoretic accumulation of DNA in the vicinity of the electrodes. For this reason, not the absolute capacitance C but its change in $(d/dt)C$ times $(d/dt)C$ was taken as a measure of DEP response. This corresponds to what Bakewell and Morgan²⁰ determined as the initial dielectrophoretic collection rate. $(d/dt)C$ is calculated from the difference in capacitance before and after the 8 s long DEP phase as $(d/dt)C=\Delta C/\Delta t$ [Fig. 3(b)]. For each frequency these values were averaged and then plotted as a spectrum [Fig. 3(c)].

In earlier studies we found that in some cases, the electrodes were damaged, particularly after application of low frequencies. Therefore, in this work, all measurements were started at the high frequency end of the spectrum. Above that, each electrode was inspected microscopically after the experiment. In no case, there was any sign of damage.

Simultaneous electrical and optical observations of DEP were performed on 24 kbp λ -DNA fragments at 1 MHz (Fig. 4). A good agreement between the courses of capacitance and fluores-

FIG. 4. Time course of dielectrophoretic collection of 24 kbp DNA at constant frequency of 1 MHz. Green dotted line: Capacitance change (d/dt) C ; red dashed line: Absolute capacitance C ; blue solid line: Intensity of simultaneously observed fluorescence.

cence intensity was found. During the first 4 min, both curves exhibited an initial drop followed by a gradual increase and then a final reduction after about 50 min. The determined capacitance change (d/dt) C as defined above was strongly negative at the beginning complementing well the capacitance and fluorescence course. After that it remained rather constant with a gradual decrease from 0.05 to 0.03 pF/s. While the cause of the initial drop is unclear, the apparent increase in DNA concentration close to the electrodes is a consequence of the expected accumulation of DNA near or at the electrodes. The final decrease in fluorescence usually would have been explained by photobleaching of the chromophores. However, because of the analogous trend of the capacitance, there must be a different explanation. Possibly more and more DNA is adsorbed at surfaces, which would lower the concentration of available DNA. This interpretation is supported by the nearly steady decrease in measured (d/dt) C . Whether the reported adhesion of DNA to aluminum³³ leads to a decreased (d/dt) C by adhesion to the electrode surface, especially to that of the inactive electrodes, or to a rise of the apparent response due to adhesion exactly at the edges of the active electrodes where the influence on capacitance should be strongest is presently unclear. Purpose built electrodes with a less adsorbing surface and a smaller inactive area could help to clarify this question.

Typically three spectra were acquired for each sample [Fig. 5(a)] starting at 3 MHz. A fourth

FIG. 5. (a) Typical (d/dt) C spectra. Three dielectrophoretic spectra have been determined starting at 3 MHz, plus a final 3 MHz value, from the capacitance time course shown in (b).

FIG. 6. (a) Dielectrophoretic spectra of 5 DNA fragments of 100 bp, 500 bp, 7.2 kbp, 24 kbp, and 48 kbp length, respectively, at a concentration of 70 $\mu\text{g/ml}$. (b) Part of (a) with enlarged $(d/dt)C$ scale.

measurement at 3 MHz served as an additional check for unintended changes. For nearly all samples, the response decreased from round to round. While the first spectrum was significantly stronger than the following ones, spectra 2 and 3 usually differed only slightly. Therefore, for the remaining evaluations the first spectrum is usually not taken into account. The temporal course of the absolute capacitance [Fig. 5(b)] shows an asymptotic base line increase due to DNA accumulation, which was to be expected due to $(d/dt)C$ being positive for all frequencies. This accumulation of DNA could be the cause for the apparently decreased response reducing the “dynamic range” of the method. Figure 5(b) is suggestive in this sense showing an upper limit for C of 930 pF in this experiment. However, this explanation appears somewhat simplistic since this effect also appeared at lower DNA concentrations, although less pronounced.

Dielectrophoretic spectra of the five different DNA species studied (100 bp, 500 bp, 7.2 kbp, 24 kbp, and 48 kbp) are depicted in Fig. 6. They represent the averages of a total of 46 spectra with 4–12 samples per DNA species, all at a DNA concentration of 70 $\mu\text{g/ml}$. For all DNA fragments, maximal response was found at around 3–10 kHz. With a minimum between 100 and 300 kHz, the response increased again to higher frequencies. The low frequency behavior corresponds very clearly to the findings of Green *et al.*³⁴ and Yokogawa *et al.*³⁵ who explained it by an ac electro-osmotic flow. This leads to accumulation of material above the electrode surface^{9,36} and to depletion between the electrodes, while DEP preferentially leads to accumulation at the electrode edges and in the electrode gaps. Both mechanisms increase the concentration of macromolecules close to the electrodes and, hence, change the measured impedance in the same direction. Therefore, the present setup cannot distinguish directly between ac electro-osmosis and DEP. Still, this could be circumvented in the future by a different electrode design, e.g., by electrical isolation of most of the electrode surface or by additional isolating constrictions.³⁷ The maxima of $(d/dt)C$ vary with DNA species between 0.17 and 0.53 pF/s without any obvious dependence on molecular weight. Since DNA concentration in weight per volume was the same for all species, within an estimated error of $\pm 20\%$, one might have expected identical responses since ac electro-osmosis primarily affects the fluid, which then carries away the DNA molecules independently of their size. However, the identical concentrations of DNA of different lengths should result in different capacitance values since the polarizability of DNA per base pair is not a constant value. For example, Tuukkanen *et al.*²⁴ reported a decreasing polarizability per base pair, for fixed frequencies, with increasing molecular weight up to around 5 kbp. In a detailed discussion, these authors explained the relatively high specific polarizability of short DNA fragments as being caused by their mechanical stiffness since the persistence length of double stranded DNA is in this range with about 130–230 bp.^{24,38} Above this size, specific polarizability was found to rise again, which

FIG. 7. Capacitance change $(d/dt)C$ as a function of DNA concentration for 24 kbp DNA. Lines represent linear regression of data at each frequency.

is in good agreement with the findings of this work. The relatively strong response of λ -DNA and its fragments in the high frequency part of the $(d/dt)C$ spectra could be the consequence of a potential additional mechanism. With contour lengths of 8 and 16 μm , respectively, these DNA strands are able to span three to six electrodes simultaneously. This might lead to increased interaction forces or interaction probability. For the shorter DNA species, $(d/dt)C$ spectra exhibit a plateau above 300 kHz. This is in good agreement with data from similar work on 3 kbp DNA (Ref. 32) and from fluorescence microscopy.²⁴ In other studies, a decrease in DEP response has been found for high frequencies²⁰ leading to even negative values above 1 MHz (Ref. 35) or 10 MHz.²³ Possible causes for this seeming inconsistency are, e.g., parasitic inductances that increasingly attenuate the driving voltages at higher frequencies and differences in medium conductivity that strongly influences the crossover frequency.

In most cases, the change in the base line, i.e., the gradual increase in C , as a consequence of DNA accumulation due to positive DEP, has no measurable impact on $(d/dt)C$. This follows from the nearly constant value at 3 MHz in Fig. 5(a) as well as from comparing the individual $(d/dt)C$ values from within one measuring period, e.g., from $t=1680$ s to $t=1800$ s [Fig. 3(a)]. However, extreme changes such as the steep decrease in C after the field application at 3 kHz [$t > 1800$ s, Fig. 3(a)] have a significant impact on the evaluated $(d/dt)C$ data. Therefore, the true $(d/dt)C$ values at 1 kHz are presumably much higher than shown. To correct this in future studies, the field application should be interrupted for a while when the frequency is changed. Such an interruption should last for about the characteristic time of this decay. In a similar study³² this has been determined to about 30 s for slightly different electrodes.

In a further set of experiments, the concentration of all DNA species was varied. The results for the 24 kbp λ -DNA fragments are shown in Fig. 7 for each frequency. Corresponding to Fig. 6, highest $(d/dt)C$ values occurred at 3 kHz, 10 kHz, and 3 MHz for concentrations between 0.12 and 70 $\mu\text{g/ml}$. These frequencies also gave the best correlation in a linear regression of the double-logarithmic plot with r^2 around 0.9. Slopes between 0.48 and 0.60 point toward a square root dependence between concentration and capacitance changes. These data compare well with those of Macanovic *et al.*²⁷ who used impedance measurements at 100 kHz to measure the concentration of DNA between 0.1 and 2 $\mu\text{g/ml}$. Still, their electrodes were about 200 times larger requiring 100 μl sample solution while in this study, only 2 μl were needed. For the other DNA species, similar correlations of the $(d/dt)C$ concentration curves were found, although they were noisier. The lower detection limit was determined—as in Ref. 27—to 0.1 $\mu\text{g/ml}$. The

FIG. 8. Absolute capacitance values as a function of DNA concentration after completing three DEP spectra for 100 bp, 7.2 kbp, and 24 kbp DNA length.

dependence of the final capacitance C after each experiment on DNA concentration is depicted in Fig. 8. For clarity, only the results for 100 bp, 7.2 kbp, and 24 kbp are shown. The large response of the 24 kbp fragment in the spectra of Fig. 6 translates to the highest slope and best correlation between final capacitance and DNA concentration. This evaluation leads to a detection limit, which is an order of magnitude higher than when exploiting $(d/dt)C$.

IV. CONCLUSION

The combination of impedance measurements with dielectrophoretic collection has been demonstrated to be able to serve at least two different purposes. First, the concentration of macromolecules can be determined without any chemical modifications of the analyte and detection is improved as compared to impedance measurements alone. Although electrical sensing only gives limited information about the type of molecules detected, specificity can be readily attained in microfluidic systems by, e.g., functionalization of the electrode surface with binding molecules such as antibodies or by combination with chromatographical methods. In contrast to optical methods, there is, in principle, no lower limit—except signal-to-noise ratio—in geometrical resolution. Thus, detection of single nanoparticles and even molecules³⁹ appears feasible. Second, the dielectrophoretic response of a wide range of nano-objects can be investigated rather easily, which should help to gain more insight into the mechanisms controlling this interaction. Hence, systematical improvement of DEP as a submicroscopical tool is greatly facilitated. Improvements in the experimental design concerning, e.g., the timing and the electrode layout should further increase sensitivity and temporal resolution.

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