

Orientation and Positioning of DNA Molecules with an Electric Field Technique

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Abstract

A controlled handling of single molecules is essential for the fabrication and the investigation of devices based on molecules. We present here the implementation of an electric field based method used to manipulate DNA molecules by means of lithographically patterned metallic electrodes. We optimized the geometry of the lithographic structures to favor a precise positioning of the molecules via dielectrophoresis. This process is combined with an orientation of the molecules

parallel to the electric field lines due to their induced dipole moment. The relatively high polarizability of the DNA molecules in solution is essential to achieve these manipulations. We expect this method to be softer than the stretching of molecules using a receding meniscus. The visualization of the molecules was achieved using fluorescence microscopy.

Introduction

The need for a controlled handling and manipulation of single molecules has drastically increased over the past years. This is due in particular to the enhanced interest for the exploration of the physical properties of biological systems and for the realization of molecular electronics devices. Besides Atomic Force Microscopy and Scanning Tunneling Microscopy, a variety of techniques has been used including electric, magnetic and optical traps allowing to move and position nano-scale objects and molecules (see for instance [1-3]). We present here an electric field based technique for the orientation and precise positioning of DNA molecules on silicon devices. The technique was designed to further investigate the recently debated question of the electric conduction properties of DNA molecules (see e.g. [4] and references therein). We focus here on the technique itself.

The double-stranded DNA molecule is a long macromolecule consisting of two strands of deoxyribonucleotides held together by hydrogen bonding. Under slightly basic conditions, the phosphate groups within the backbone deprotonize and the DNA molecule is negatively charged. This charge leads to the formation of a counterion cloud surrounding the molecule to an extension given by the

Debye length. A DNA molecule has essentially no net permanent dipole moment since the two helices forming the double-strand DNA point in opposite directions. However, the counterion cloud can be displaced in the presence of an electric field and it is expected to strongly increase the polarizability of the molecule (ionic polarizability). The suspended molecule can then be treated as a dielectric medium of given volume and shape placed in a continuum solution of different dielectric properties [5].

Electric manipulations of DNA molecules in microfabricated structures based on the induced dipole moment of the molecules have been carried out since the last decade [2]. Such devices allow to work with relatively high applied electric potentials (in the MV/m range) using low voltage sources. Furthermore, due to the small size of the structures, sample cells with small volumes (down to a few picoliters) can be used. So far, a relatively high number of molecules was manipulated within such devices [2, 6 and 7]. The design of the electrodes in our devices allows to achieve a controlled fine positioning and anchoring of a small number of DNA molecules.

Experimental

Microfabrication

The electrodes (Figure 1 and 2) were fabricated using electron beam lithographic techniques. After a usual cleaning

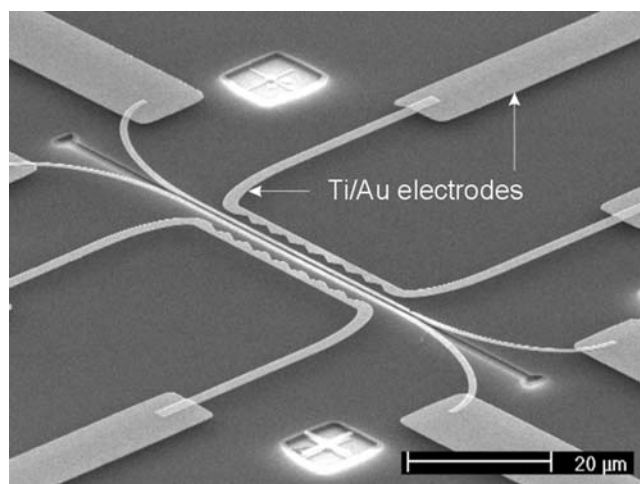


Fig. 1. Electron microscope picture of one device fabricated on a $\text{Si}_3\text{N}_4/\text{SiO}_2/\text{Si}$ wafer. The saw-tooth electrodes tip angle is about 120° . The gap between the central electrodes is about 300 nm wide and a 700 nm deep etching in the SiO_2 layer was performed to allow the investigation of suspended molecules.

(sonication in organic solvents and oxygen plasma treatment), p-doped Si/SiO_2 prime wafers were coated with a standard positive resist (PMMA, Allresist) and an electron beam writer (JSM-IC 848, JEOL) was used to expose the structures. After development, the metallic electrodes (typically 5 nm Ti adhesion layer and 40 nm Au) were deposited using a high-vacuum evaporation chamber (PLS 500, Balzers). A final lift-off step concluded the process. The fabrication was carried out on 20 mm x 20 mm wafer pieces comprising each a set of four devices. A scanning electron microscope (XL30-FEG, Philips) was used to examine the structures. In order to test free-standing molecules, some of the devices were fabricated on $\text{Si}_3\text{N}_4/\text{SiO}_2/\text{Si}$ wafers allowing the fabrication of an underetched gap in the SiO_2 layer. First, a groove was etched in the Si_3N_4 layer (210 nm) using reactive ion etching (0.025 mTorr of a $\text{CHF}_3 : \text{O}_2$ mix in a ratio of 34:4). The SiO_2 layer was then etched using buffered HF (28ml HF 40 % diluted in 170ml H_2O and mixed with 113g NH_4F). Figure 1 shows an electron microscope picture of such a structure. The width and depth of the gap are respectively 300 nm and 700 nm.

Fluorescence Microscopy

The DNA molecules (λ -DNA, 48.5 kbp, 16.5 μm long, Boehringer Mannheim) were stained with a fluorescent dye (YOYO[®]-1 iodide, Molecular Probes). The dye and DNA amounts were adjusted in order to obtain an average ratio of one dye molecule per five base pairs of DNA. For our experiments, we prepared a 1 $\mu\text{g}/\text{ml}$ stained DNA solution in MES (2-[N-morpholino]ethanesulfonic acid hydrate, Sigma) buffer at pH 4.1.

The fluorescence images were realized with a fluorescence microscope (BX51, Olympus) equipped with a 100 x/NA 1.35 oil immersion objective and a cooled CCD camera (Orca II, Hamamatsu). In order to reduce bleaching, the molecules were illuminated only during CCD exposures (20ms to 1s). The light of a vapor mercury lamp was filtered through an excitation filter (BP, 460nm-490nm) and a dichroic mirror (505nm). Fluorescence light was collected on the CCD camera through the dichroic mirror and an emission filter (HP, 510nm).

Application and Discussion

Principles of the Technique

In the presence of an electric field \vec{E} , a DNA molecule in solution will bear an induced dipole moment given by $\vec{p} = \alpha V \vec{E}$ where α is the polarizability of the molecule per unit volume

and V is the volume. The polarizability depends on the permittivities of the molecule and of the solution. The counterions cloud is expected to strongly enhance the polarizability of the molecule and the induced dipole moment will thus depend on the solution used and in particular on its ionic strength and pH. However, working with solutions containing a high concentration of ions would facilitate electrochemical reactions at the electrode-solution interface. Such a situation is detrimental to the experiment and can cause irreversible damages to the electrodes due to the relatively high fields applied. An advantage of the technique used here is that it allows working with *ac* electric fields in the frequency range of a few kHz up to a few MHz. Combining this with relatively low-conductivity buffers (typically below $100 \mu\text{S cm}^{-1}$) allows to limit the voltage drop at the electrode-solution interface and helps reducing electrochemical reactions.

The manipulations of the molecules are based on the interaction of the induced dipole moment and the applied electric field. The translational motion of the molecules is caused by the application of a non-uniform electric field to the solution (dielectrophoresis, see for instance [9]) which is realized in our case by the saw-tooth electrodes. In a non-uniform field, a neutral object of polarizability α and volume V will undergo a force proportional to the square of the applied field \vec{E} and given by $\vec{F}_d = \alpha/2 \cdot V \vec{\nabla} |E|^2$. For a dielectric ellipsoid of volume r^2l (r : radius, l : length) and permittivity ϵ_2 immersed in a medium with permittivity ϵ_1 , the force at equilibrium can be estimated by [8]

$$\vec{F}_d \approx r^2 l \epsilon_1 \epsilon_0 \vec{\nabla} |E|^2 \quad (1)$$

as long as $\epsilon_2 \gg \epsilon_1$ which is expected for DNA in an aqueous buffer. A rough estimate of the electric field value E_{th} necessary to overcome thermal fluctuations can be obtained by integrating Eq. 1 and comparing the result to $k_B T$. Since the DNA molecule is a long, flexible polymer, we will consider here the force exerted on one segment of the molecule. Taking the permittivity of water and using a radius of 1 nm for a λ -DNA molecule and a persistence length of 100 nm, we obtain

$E_{th} \lesssim 10^7 \text{ V/m}$ showing the necessity to use relatively high

electric fields. In this particular case where the dielectric object is more polarizable than the medium, the force will be directed towards the highest intensity point of the electric field (positive dielectrophoresis).

The interaction of the induced dipole moment with the applied electric field will also cause electrically non-symmetrical molecules to sense a torque $\vec{T} = \vec{p} \times \vec{E}$. High aspect-ratio molecules will thus tend to align themselves with their longest axis parallel to the applied field. In the case of a long flexible molecule such as DNA, the orientation effect is

expected to take place for every segment of the molecule, resulting in an uncoiling of the molecule to its full length.

Trapping

Figure 2 shows the trapping of DNA molecules upon the application of an electric field of 10^6 V/m at 1MHz between the two saw-tooth electrodes. The molecules are attracted (positive dielectrophoresis) to the high field intensity region and partially oriented across the electrodes. The orientation effect is clearly visible in Figure 3. The technique operates between a few kHz and a few MHz with a better efficiency of the trapping process between 100kHz and 1MHz. We used here a 1mM MES buffer at a pH of 4.1 The conductivity of the buffer was about $70 \mu\text{S cm}^{-1}$. In case of a strong accumulation

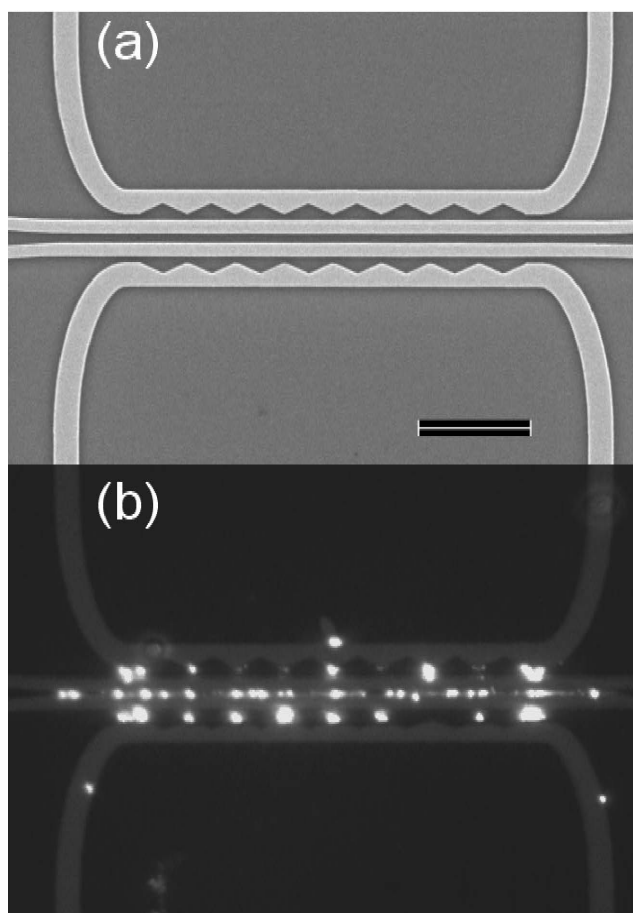


Fig. 2. (a) Electron microscope picture of one device without underetched gap. The saw-tooth electrodes tip angle is about 120° . The scale bar is $10 \mu\text{m}$. (b) Fluorescence image of the same device showing DNA molecules trapped between the tips of the saw-tooth electrodes. The fluorescence is partially quenched over the central electrodes.

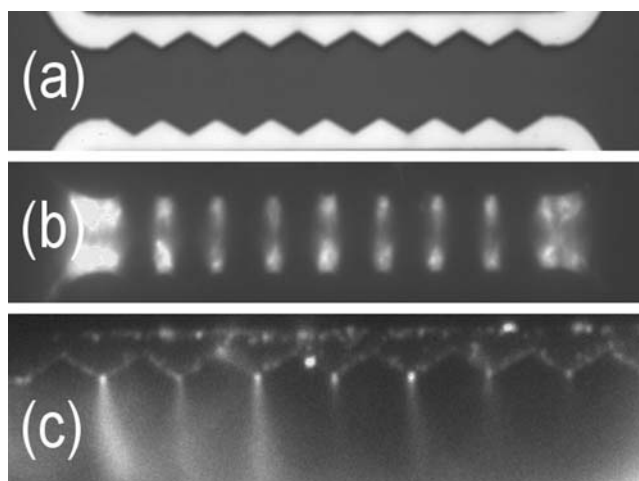


Fig. 3. (a) Detail of a light microscope picture (white light) of one device (no central electrodes here). The vertical separation between the two electrode tips is about $4\ \mu\text{m}$. (b) Fluorescence image of the same device showing DNA molecules trapped between the tips of the saw-tooth electrodes. (c) Detail of DNA molecules trapped at the electrode tips (device similar to that shown in Fig. 1). The blurry appearance of the molecules is due both to their partial extension away from the surface and to their motion during the CCD exposure time (100ms). The horizontal distance between two tips of the electrodes is about $4\ \mu\text{m}$.

of molecules between the central electrodes (floating during the trapping process), a negative potential of a few hundred mV applied for a short time to the central electrodes allows to move the DNA molecules away from the surface very easily.

Due to the relatively high electric field density, any *dc* or low-frequency current in the solution would lead to strong electrochemical reactions taking place primarily at the electrode-solution interface. The fact that the technique works efficiently at relatively high frequencies is an invaluable advantage since it helps limiting such electrochemical effects by lowering the potential drop at the electrode-solution interface. To prevent any low-frequency contribution, a high pass RC filter was mounted on the saw-tooth excitation electrodes.

The total volume of the fluid cell was typically of a few μl resulting in a liquid layer thickness of about $10\ \mu\text{m}$. This is a relatively small volume and Joule heating of the solution can become critical at high applied electric fields. Indeed, the Joule loss can be estimated to about $7\ \text{W}\ \mu\text{l}^{-1}$ for an applied field of $10^4\ \text{V/cm}$ and a buffer conductivity of $70\ \mu\text{S cm}^{-1}$. In adiabatic conditions, this would result in an almost instantaneous evaporation of the liquid within the fluid cell. Since the electric field is very intense only in a limited volume around the electrodes and since the heat is dissipated in the whole cell, the overall temperature raise remains acceptable

[13]. A rough estimate considering a heated volume of $10\ \mu\text{l}$ and a total cell volume of $2\ \mu\text{l}$ yields a temperature increase of 8mK per second. A good thermal coupling of the device under test to a sample holder further helps limiting heating effects.

Figure 3 shows a more detailed view of the trapping process between two saw-tooth electrodes without central electrodes. The first optical microscope picture (Figure 3 (a)) shows the electrodes in the absence of DNA molecules. Figure 3 (b) is a fluorescence image of the same device with DNA present in the fluid cell at a concentration of $1\ \mu\text{g/ml}$ and with an applied field of 4V (1MHz) between the electrodes. The vertical distance between the two electrode tips is about $4\ \mu\text{m}$. Figure 3 (c) shows finally a closer view of the electrode tips with DNA molecules anchored at their top. The typical time scale for the DNA molecules to reach the electrodes once the field is applied is in the range 0.1s to 1s when starting from a solution at rest (no flow). The structure used in this case was similar to that from Figure 1. The orientation effect appears here more clearly.

The molecules manipulated with this technique undergo a stretching force while the field is applied. The force on one molecule can be estimated in a rough approximation by the sum of the (opposite) electrostatic forces $F_s = qE$ exerted on both ends of the molecule. The charge q is simply related to the induced dipole moment by $p = ql$ where l is the length of the molecule. A dipole moment value of $10^{5\text{D}}$ was obtained for DNA molecules with a molecular weight of about 3MDa (about $1.5\ \mu\text{m}$ long) [10]. Using these values, we obtain an estimate of 0.2pN for the force with an applied field of $10^6\ \text{V/m}$. The stretching force appears thus to be at least one to two orders of magnitude below the forces sustained by the molecules when using a receding meniscus where their elongation can exceed 50% (see e.g. [11]). The increase in length of a λ -DNA molecule of length $l_0 = 16.5\ \mu\text{m}$ due a force of 0.2pN can be estimated to about 10nm using Hooke's law $F_s = EA(l-l_0)/l_0$ where E is the Young modulus [12] and A the cross sectional area. We thus believe that this method used in proper conditions (solution, applied field) can be much softer than molecular combing.

In conclusion, we have presented a silicon-based device allowing a controlled, precise positioning and capture of a relatively small number of DNA molecules in free-flow. We expect that such devices will be useful for the handling of minute amounts of molecules in a variety of experiments involving DNA separation, amplification or detection. The further development of integrated systems such as biosensors or "lab-on-a-chip" devices for biochemical experiments requiring several steps will certainly make use of high intensity electric fields for an efficient handling of the molecules involved.

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