Label-free Immobilization of Nano-particles on Silicon based Electrodes for Single-biomolecule Studies

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Abstract: Dielectrophoresis (DEP) is an established method for the spatial manipulation of microscopical particles. We demonstrate the temporal and permanent immobilization of polystyrene nanoparticles and protein molecules with sizes ranging from 4 nm to 500 nm. For this, regular arrays of silicon based nanoelectrodes were developed with tip diameters of 10 nm and 50 nm. No chemical modifications of molecules, particles or surfaces were needed. This opens up potentially important applications of DEP in biosensing and cell research.

1 INTRODUCTION

There is a growing need in lab-on-a-chip systems and similar biodevices for spatial manipulation of nanoparticles like concentrating, immobilizing, orientating and aligning. The manipulation should be performed on a large number of objects simultaneously. AC electrokinetic methods like DEP have been successfully applied for some decades by exploiting alternating electric fields between microelectrodes. In the case of non-uniform fields, polarizable particles get immobilized on top or at the edges of the electrodes, as illustrated in Fig. 1.

So far, most of the research work performed on DEP has been done by using metal electrodes (Widdershoven 2010, Martinez-Duarte 2012). The continuous downscaling of CMOS minimum feature sizes provides great opportunities. By adapting the typical electrode dimensions to the objects' size, it has become possible to manipulate even single objects like viruses and proteins on metal electrodes (Yamamoto 2007, Pethig 2010, Diao 2011 Nakano 2013). Nevertheless, the dimensions of CMOS metal electrodes are still in the range of 100 nm (ITRS 2012 update), which doesn't fit to nanoparticles like proteins with diameters of less than 10 nm (Hölzel 2005). A modern approach to optimize the interaction between particles and electrodes is the use of doped triangular shaped silicon as electrode material.

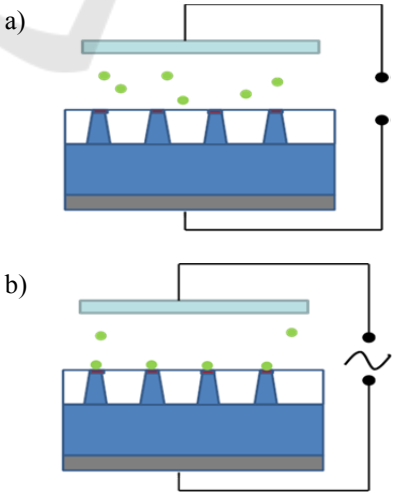

Figure 1: Dielectrophoresis: Schematic illustration of the experimental setup. a) Nanoobjects are suspended in water b) By applying inhomogeneous AC electric fields, electrical forces act on polarizable particles. These forces lead to a local particle accumulation. Distance between top electrode and electrode surface is about 100 µm.

2 EXPERIMENTAL

Cone-shaped nanoelectrodes were fabricated in a

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standard CMOS process line by using reactive ion etching (RIE) process techniques (Mehr 1996). The minimum tip radius is about 1.5 nm. The electrodes are embedded in a SiO_2 matrix and the diameter on top of the tips can be increased by chemical mechanical polishing (CMP), as shown in Fig. 2. The process flow is completed with silicidation of the tip surface (CoSi). The total number of electrodes amounts to about 100.000 per array (Fig. 3).

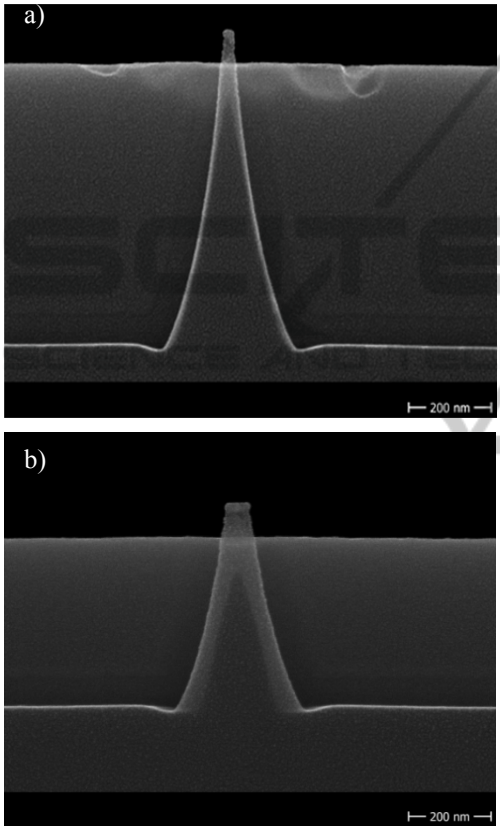

Figure 2: Cross-sectional scanning electron microscope (SEM) image of plasma etched electrodes with a) 10 nm and b) 50 nm diameter at the top of the silicon based electrodes.

The DEP force acting on the particles is directed along the gradient of the electric field. Therefore, the implementation of a steep electric field gradient is required to maximize the DEP force acting on the particles.

The simulation (Fig. 4) shows the largest electric field gradient and, hence, the strongest DEP force on top of the 10 nm tip electrode. For the 50 nm top surface, the strongest force is localized at the edges of the electrode, leading to a ring-like alignment. Obviously, for single molecule immobilization, the tip diameter has to be minimized.

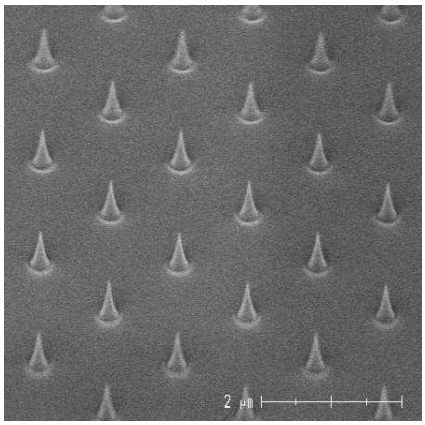

Figure 3: SEM image of the plasma etched nanoelectrode array.

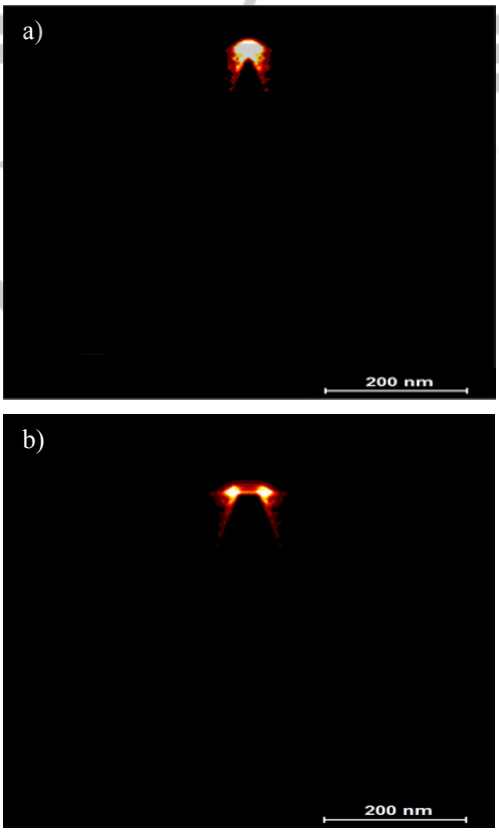

Figure 4: Simulation of the electric field gradient grad $|E|^2$ above the nanoelectrodes with a) 10 nm and b) 50 nm tip diameter. Maximum field gradient (white) is close to the tip.

To demonstrate the permanent immobilization of a biomolecule on an electrode array (Fig. 5), we used the fluorescently labeled bovine serum albumin (BSA), which is a protein of prolate ellipsoidal shape (14 nm x 4 nm x 4 nm (Squire 1968)).

Typically, these experiments were carried out at about 10 kHz with 5 to 10 V_{RMS} for periods of some seconds to minutes. By choosing the optimum operating conditions, the immobilization of nanobeads with diameters of 200 nm is finalized within a few minutes, as illustrated in Fig. 6.

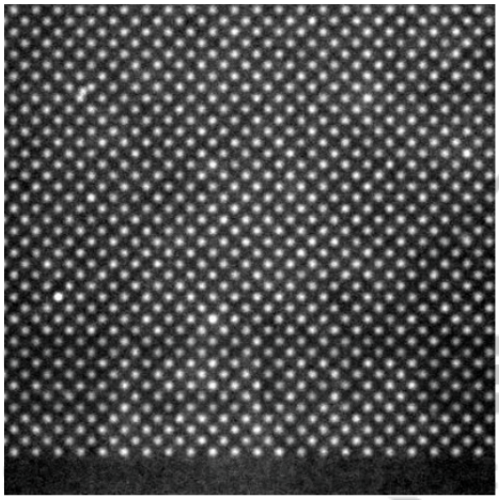

Figure 5: DEP field induced immobilization of the fluorescently labeled protein BSA on nanoelectrodes after field application of 10 kHz and 7 V_{RMS} for 10 min.

The distribution of fluorescence intensity of immobilized nanobeads with diameters of 20 nm on electrode tips with diameters of 50 nm is quite broad, as shown in Fig. 7a. That means, ensembles of numerous nanobeads were immobilized on each electrode tip.

However, by applying this system to objects that were slightly larger than the electrode tips, we were able to achieve a proper 1:1 ratio between particles and tips, as illustrated in Fig. 7b. That means, exactly one nanobead was placed on each electrode tip.

This opens a completely novel approach to single-molecule investigations on large ensembles. This deterministic control of local particle numbers in aqueous solutions demonstrates the importance of reducing the typical electrode dimensions to 10 nm and less.

In addition, one has to consider that alternating electric fields lead to Joule heating in the liquid medium (Seger-Sauli 2005). Local temperature raises could cause thermal stress, cell damages and protein denaturing. Therefore, we measured the heating of the medium close to the electrodes at 1 MHz and 9 V_{RMS} as a function of electrode diameter. The temperature variation was detected by exposing the thermo-dependent fluorescent dye

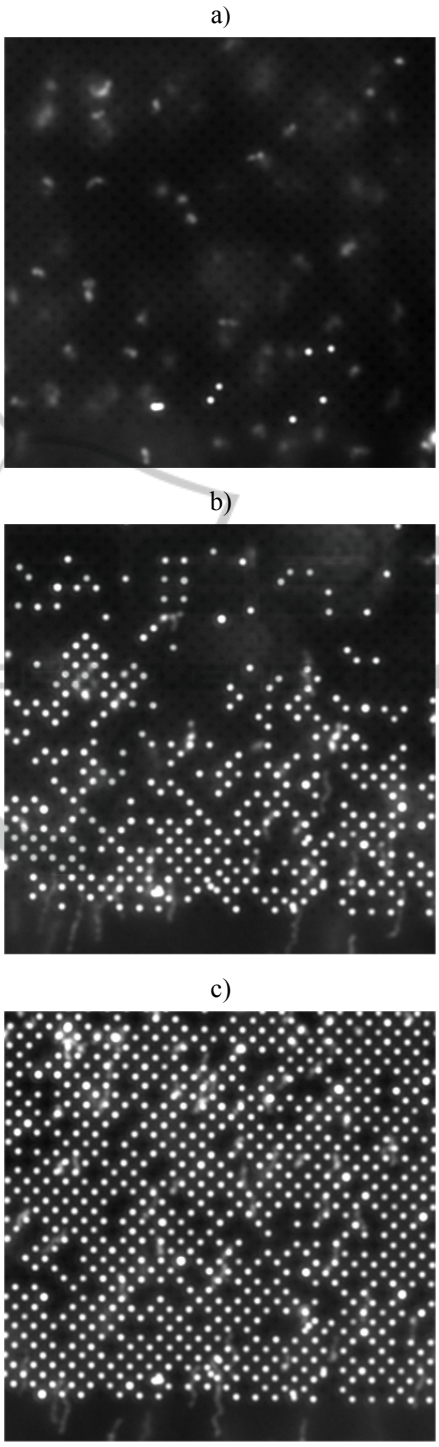

Figure 6: Top view microscopic images through the transparent top electrode of 200 nm fluorescent polystyrene nanospheres in water on the nanoelectrode array. a) Before field application, b) after field application at 17 kHz and 8 V_{RMS} for 12 s and c) after 60 s field application.

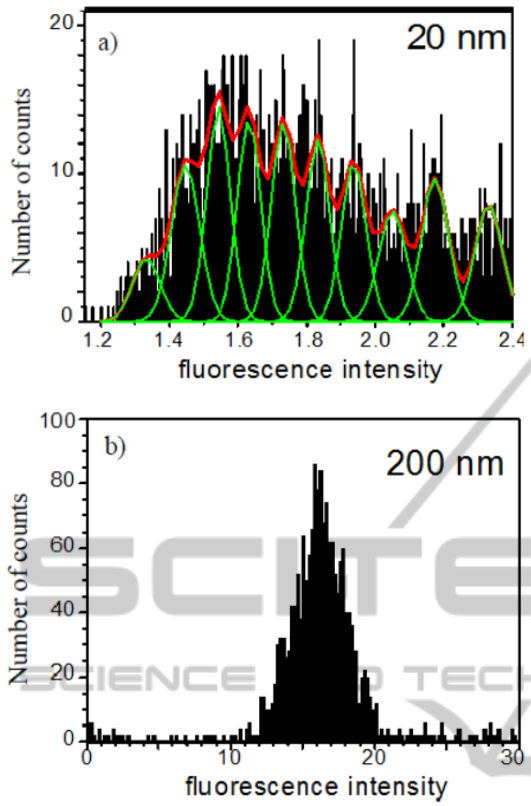

Figure 7: Fluorescence intensity distribution of dielectrophoretically immobilized polystyrene nanospheres. Bead diameters are a) 20 nm and b) 200 nm. Electrode diameter is 50 nm. a) For particles smaller than the electrodes, accumulation of approx. 1 to 15 particles per electrode tip occurs. b) 200 nm spheres are immobilized as singles.

rhodamine B to the test set-up. Its fluorescence intensity decreases with temperature by about -1.2%/K

As shown in Fig. 8, temperature rises within 220 s by 5 °C for 10 nm tip diameter and by 6 °C for 50 nm tip diameter. This improvement by smaller electrodes can be explained by the corresponding reduction of the volume carrying high current densities. Above that, the increased surface-to-volume ratio enhances heat dissipation from the electrodes.

As illustrated in Fig. 9, the proposed silicon-based technology for immobilization of nanometer sized molecules by cone-shaped nanoelectrodes opens a new CMOS compatible platform for the analysis of single proteins and their functions.

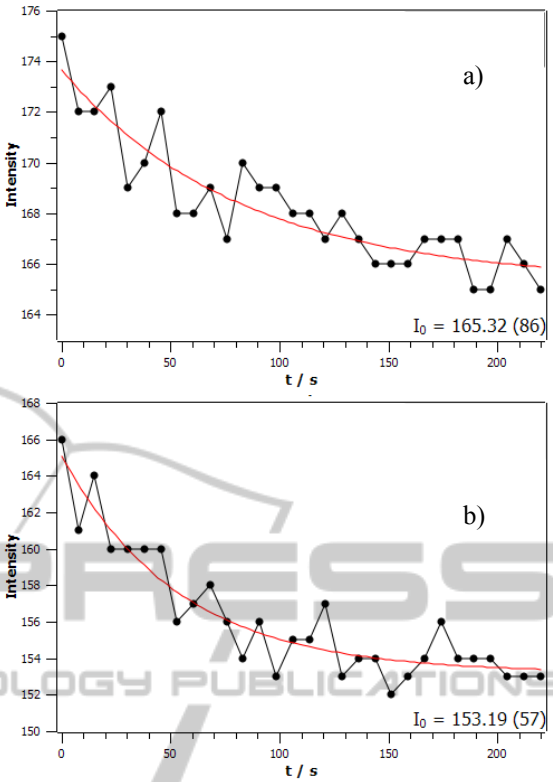

Figure 8: Intensity characteristics of the thermo-dependent fluorescence of rhodamine B in order to evaluate the temperature increase in the liquid medium above the nanoelectrodes with a) 10 nm and b) 50 nm tip diameter.

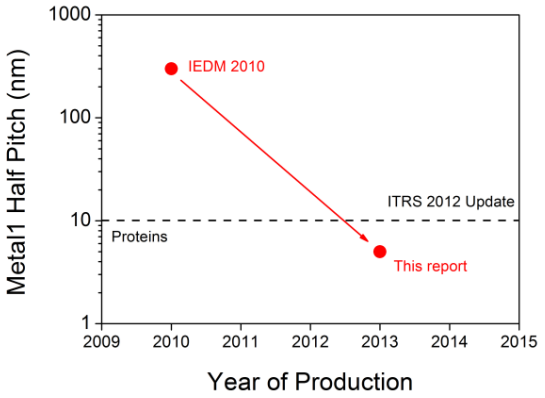

Figure 9: CMOS Metal1 half pitch roadmap (ITRS 2012) compared to typical dimensions of proteins.

3 CONCLUSIONS

We demonstrated the electrically controlled immobilization of biomolecules and nanospheres using silicon based nanoelectrode arrays. The nanoelectrodes with tip sizes of 10 and 50 nm were

fabricated by reactive ion etching (RIE) techniques in a standard CMOS process line. Electric field distributions were calculated. Temperature changes within the device were determined by optical microscopy to about 5 °C, which is well compatible with biomedical applications.

Nanospheres with diameters of 20 nm and 200 nm suspended in water were immobilized at the electrode tips. For particles larger than the electrodes, immobilization of single objects was demonstrated. The procedure was performed within a few tens of seconds. Any chemical modifications of suspended particles, dissolved molecules or surfaces could be avoided.

The demonstrated use of silicon based nanoelectrode arrays for the dielectrophoretic immobilization of particles and molecules opens a novel way for nanoparticle separation and for the preparation of miniaturized biosensors.

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