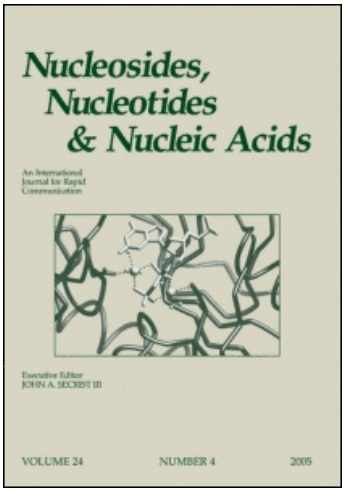

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A Fluorescence Detection and Selection Device for Dna-Molecules Bound to Microspheres

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A FLUORESCENCE DETECTION AND SELECTION DEVICE FOR DNA-MOLECULES BOUND TO MICROSPHERES

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ABSTRACT A device for the detection and selection of DNA-molecules bound to microspheres has been realized. It is based on a quartz chip with a capillary flowsystem, and confocal fluorescence detection of DNA. The device is to be used to identify and isolate single DNA-molecules for sequencing experiments.

1. Introduction

In a new technology for DNA-sequencing, based on single nucleotide detection, a need to detect and isolate single DNA-molecules has appeared. By linking the DNA-molecule to a solid carrier (microsphere), the brownian motion is overcome, thus allowing the molecule to be manipulated in solution. The sequencing of the molecule will then take place, consisting of an exonuclease degradation of the DNA, and subsequent fluorescence detection and identification of the individual nucleotides [1,2]. It is however of critical importance that there is only one DNA-molecule on the sphere for the sequencing principle to work. For this reason, we have developed a device that screens spheres for the existence of DNA and selects those that have only one[3]. The device is based on a microstructure chip and confocal fluorescence detection of dye-labeled DNA.

2. Experimental

In order to be able to treat beads in a confined geometry, we used capillary microstructures as a flow cell in the device. The structures are on a 2x2 cm quartz chip with an H-like capillary system (fig. 1). The branches within the system makes it possible to mix solutions coming from different cups, and to switch the flow into different branches for sorting purposes. VLSI-technology is used to fabricate the microstructures out of a quartz substrate (IMCaps), thus giving it micrometer precision [4,5].

The fabrication process involves one photolitographic step. The capillaries are defined by patterning rows of small holes in a deposited polysilicon layer. An etching step with HF-acid follows, that etches out the half-cylinder shaped capillaries (fig. 2). Finally an oxidation of the polysilicon, followed by a deposition of additional SiO_2 makes the capillary roof transparent and seals the holes. Capillaries with radii ranging from 20-50 μm have been used.

The microstructure chip was placed in a chip holder of plexiglass with connections for liquid and electrodes to the outer world. Electric fields could thus be applied over the capillary system, which could be used for electroosmotic pumping of liquid through the capillary tubes. Electro-osmotic pumping is a phenomena occuring in liquids at charged surfaces, when an electric field is applied parallel to the surface. The liquid is polarized by the surface interaction, resulting in an innermost layer of positive ions. When an electric field is applied, this layer is brought into motion in the direction of the field. In a capillary of small dimensions, the whole body of fluid is moved. Thus the flow can be directed by simply applying appropriate voltages over the capillaries[6]. In our experiments, we also had the possibility to apply small hydrostatic pressures to the tubes through the liquid connections.

DNA-samples of 217 basepair biotinylated PCR-products with approximately 5 incorporated TMR-labelled nucleotides were used. The DNA-molecules were bound to 2 μm diameter polystyrene beads (Interfacial Dynamics Corporation) having a streptavidin coating.

The fluorescence detection was made with an epi-illuminated confocal setup. The beam of an Argonlaser, operating at 514,5 nm was introduced into a microscope, focused down into the capillary, and the induced fluorescence was collected by the same objective. The fluorescence was then directed through a bandpass filter, and detected by an avalanche photon detector[7].

An original solution containing beads with DNA is introduced into the microstructure by a hydrostatic pressure. The beads are then directed to a detection spot and their fluorescence is measured. The fluorescence signal from the bead, apart from an intrinsic fluorescence of the bead itself, is proportional to the amount of DNA bound to it. When the signal from the bead is above a specified level, an electric field is turned on for a few seconds over the capillary. This

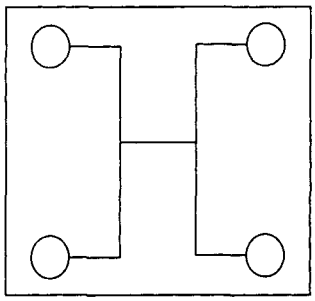

FIG. 1 Chip layout with H-shape capillary system.

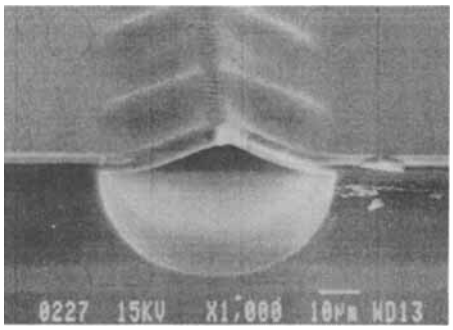

FIG. 2 SEM picture of capillary cross section

induces an electroosmotic pumping pulse that drives the bead into a selection leg. The process is automated by a computer program that monitors the fluorescence signal continuously and sets voltages at the capillary ends accordingly (fig. 3 & 4) .

Fluorescence detection experiments have been made on beads with different loadings of DNA, as well as beads with different intrinsic fluorescence. Selection experiments have been made on mixtures of beads with different intrinsic fluorescence.

3. Results and Discussion

The fluorescence measurements on beads without DNA showed that they have an intrinsic fluorescence, varying between different kinds. In our setup, undyed polystyrene beads with a streptavidin coating emitted a fluorescence corresponding to 3-4 free TMR-molecules. In diagram 1, the measured signal of naked beads taken up as they flow past the detection spot is

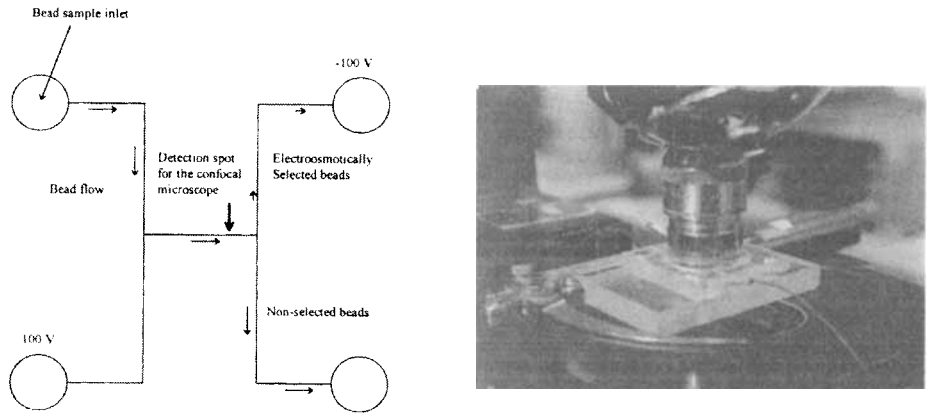

Fig. 3 & 4 The bead selection process and setup with the chip in a plexiglass holder.

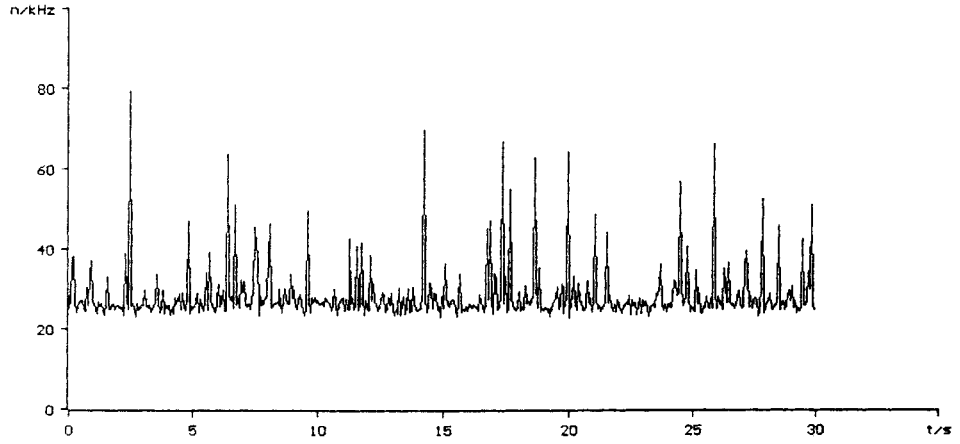

DIAGRAM 1. Fluorescence intensity peaks from beads with no loading

shown. A rather constant background level of ~25 kHz coming from the microstructure and the solution is seen, and the signals from the beads themselves are in the range of 50-100 kHz.
In diagram 2 the same kind of measurement is shown, but this time on beads with a large number of DNA-molecules attached. A difference in signal of several orders of magnitude is seen, as compared to the non-loaded beads. From measurements on free DNA in solution, we know that the signal from one molecule is approximately 100 kHz. However difficult, it would thus be possible to distinguish a singleDNA-molecule attached to a bead.

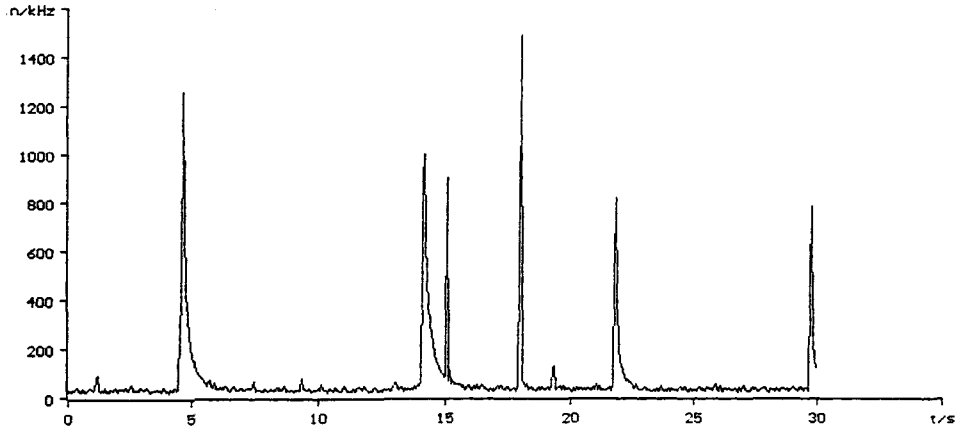

DIAGRAM 2. Fluorescence intensity peaks from beads with a large DNA loading

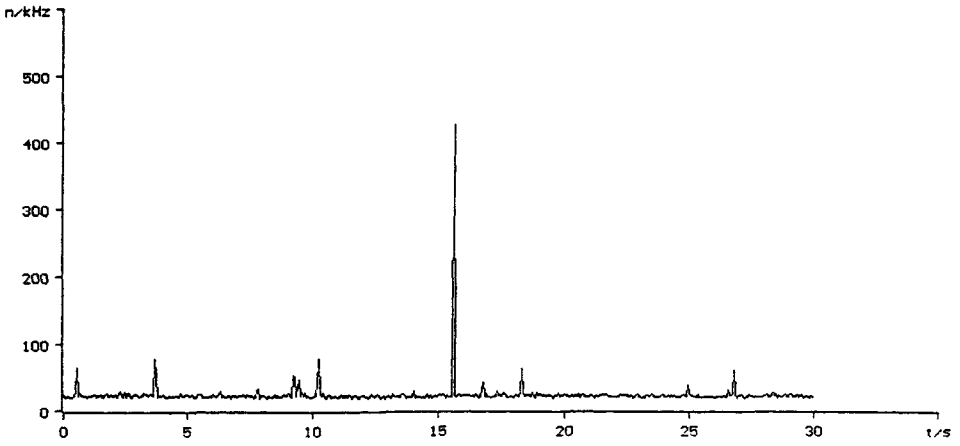

DIAGRAM 3. Fluorescence measurement during a selection experiment. The highest peak is from a bead with high autofluorescence.

Selection experiments on mixtures of beads with different autofluorescence were quite succesful. In diagram 3, a measurement taken up during a selection process is shown. The highly fluorescent bead was selected by electroosmotic pumping into one capillary leg. The beads emitting low fluorescence, together with the bulk of the liquid, was transported to the waste reservoir.

4. Conclusion

We have developed a device that automatically screens microspheres for bound DNA in a flow through system. Based on their fluorescence signal, the spheres can be selected within the

capillary system and transported to an outlet. We believe this to be the appropriate method to isolate single DNA-molecules for sequencing purposes.

5. REFERENCES

1 Rigler, R., "Fluorescence correlations, single molecule detection and large number screening. Applications in biotechnology", *J. of Biotechnology* **41**, 177-186, (1995)

2. Soper et al, "Rapid sequencing of DNA based on single molecule detection", SPIE, Optical Methods for Ultrasensitive Detection and Analysis **1435**, 168-178, (1991)

3. Holm, J., "Development of a detection and selection device for DNA-molecule carriers", M.Sc. diploma work, IMC AB - Karolinska Institute, Stockholm, (1996)

4. Kaplan W., Elderstig H., Vieider C., "A novel fabrication method of capillary tubes on quartz for chemical analysis applications", *IEEE, Micro Electro Mechanic Systems*, (1994), Japan

5. Forssén, L.; Elderstig, H.; Eng, L.; Nordling, M., "Integration of an Amperometric Glucose Sensor in a μ-TAS", *Proceedings of the μ-TAS'94 Workshop*, 203-207, University of Twente, The Netherlands, (1994)

6. Harrison, D.J.; Manz, A.; Glavina, P.G., "Electroosmotic pumping within a chemical sensor system integrated on silicon", *IEEE International Conference on Solid State Sensors and Actuators, Transducers '91*, p. 1089, 792-795

7. Mets, U. and Rigler, R. "Submillisecond detection of single Rhodamine molecules in water.", *J. Fluorescence* **4**, 259-264, (1994)