Micromachined pipettes integrated in a flow channel for single DNA molecule study by optical trapping

Cristina Rusu, Ronny van't Oever[a], Meint de Boer[a], Henri Jansen[a], Erwin Berenschot[a], Miko Elwenspoek[a], Martin L. Bennink, Johannes S. Kanger, Bart G. de Grooth, Jan Greve, Juergen Brugger[b], Albert van den Berg[c]

Applied Physics, Biophysical Techniques Group,
[a]Electrical Engineering, Transducers Technology Group, [b]NanoLink, [c]μTAS
University of Twente, MESA Research Institute, P.O. Box 217, 7500 AE Enschede, the Netherlands

Abstract

We have developed a micromachined flow cell consisting of a flow channel integrated with micropipettes. The flow cell is used in combination with an optical trap set-up (optical tweezers) to study mechanical and structural properties of λ-DNA molecules. The flow cell was realised using silicon micromachining including the so-called buried channel technology to fabricate the micropipettes, the wet etching of glass to create the flow channel, and the powder blasting of glass to create the fluid connections. The volume of the flow cell is 2μl. The pipettes have a length of 130μm, a width of 5-10μm, a round opening of 1micron and can be processed with different shapes. Using this flow cell we stretched single molecules (λ-DNA) showing typical force-extension curves also found with conventional techniques.

Keywords: micropipette, buried channel technology, powder blasting, silicon micromachining, single molecule, DNA,

Introduction

The traditional techniques of measuring molecule properties used in biochemistry are based on measuring a great number of molecules at the same time, therefore only average features are determined. Recent advances in scanning probe and optical techniques opened the way for single molecule detection and manipulation [1]. Scanning probe techniques allow the study of single molecules on surfaces, and optical techniques characterise it in complex environments.

Improvement of these research methods (e.g. accuracy, easyness of operation) can be done, for example, by using micromechanical technology [2-6]. Micromechanical structures can be developed not only aiming at applications (e.g. sequencing) but also for new investigation equipment for fundamental research [7-10].

We have developed micromachined micropipettes that can be used in combination with optical tweezers to study mechanical and structural properties of λ-DNA molecules. In the DNA molecule stretch measurements, the ends of a single DNA molecule are biochemically bound to two polystyrene beads (using streptavidin-biotin connection). The beads can be manipulated using the pipette and the optical tweezers, which is used to apply and measure the stretching force on the DNA molecule [11-13].

This paper describes the design, fabrication (using silicon technology) and testing of the micromachined flow cell. The flow cell consists of a flow channel with integrated micropipettes. Buried Channel Technology [14] is used to make the micropipettes, wet etching of glass to create the flow channel, and the powder blasting [15] of glass to make the fluid connections. The common flow channel is made by hand by gluing two microscope cover glasses together using two parafilm as spacer, in which a glass capillary pipette is integrated (see fig. 1a). It would be easier having a flow cell with the pipettes already integrated (fig 1b).

The microfabrication method has the advantages (i) to create a flow cell in which many pipettes are already integrated into a flow channel, (ii) that various shapes of the micropipettes can be obtained by using different designs and technological processes, and (iii) that reproducible dimensions of the pipettes and channel are obtainable. This makes it possible to choose for each type of experiment the best pipette shape. Having many micropipettes in a flow channel, parallel measurements on single molecules can be performed allowing rapid 'statistical' data acquisition.

In *Micro- and Nanotechnology for Biomedical and Environmental Applications*, Raymond P. Mariella, Jr., Editor, Proceedings of SPIE Vol. 3912 (2000) • 1017-2661/00/$15.00

Besides DNA measurements, these pipettes can be also used for drug delivery, for injection of small gas bubbles into a liquid flow to monitor the streamlines, and for the mixing of two liquids to study diffusion effects.

Experimental set-up

The current experimental set-up using an optical trap is a home-made microscope system and a flow cell unit that are described in more details in [13]. A laser diode (829nm, 200mW) is used for the optical trap [16]; a micrometer-sized polystyrene bead can be captured just behind the focal point of a laser beam. The laser beam from the laser diode is focused using a 100x objective with a numerical aperture (NA) of 1.2. When external forces are exerted on the trapped bead, the bead will move out of the centre of the laser beam and will cause the laser beam (transmitted through the bead) to deflect from its original position [17]. The transmitted beam is projected onto a quadrant detector and its position is used to determine the force acting on the bead in the optical trap. The exerted force on the bead is up to 70 pN, the optical trap stiffness is about 200 pN/μm, and the precision in determining the DNA molecule length is 5-10 nm [13].

The DNA used in experiments was bacteriophage λ-DNA obtained from New England Biolabs (Beverly, MA), and the 2.5 μm polystyrene beads with streptavidin coating from Bangs Laboratories Inc. (Fishers, IN). The biochemical protocol is described in [13].

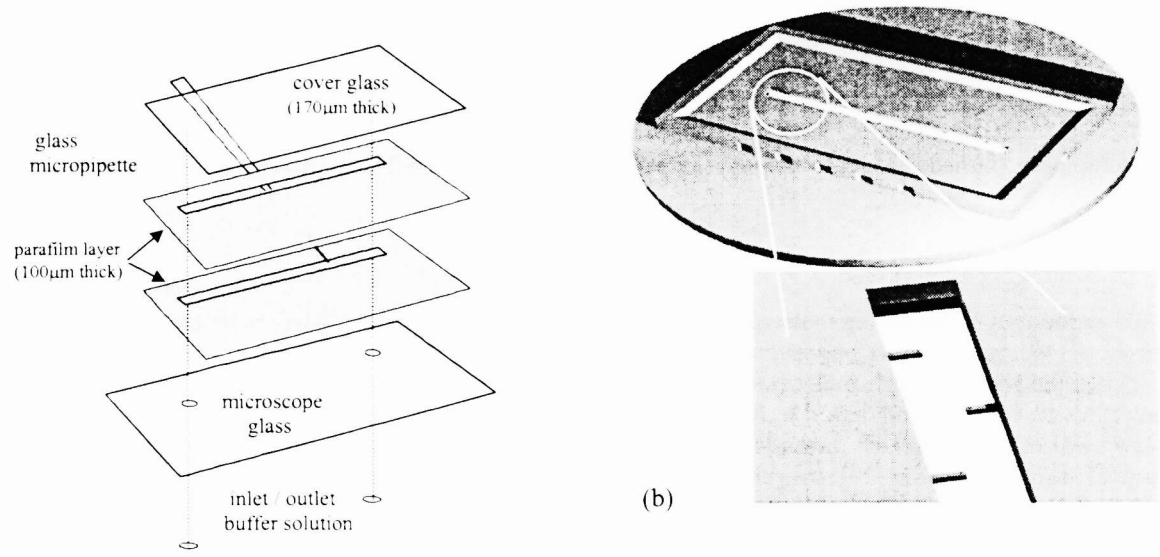


Fig. 1. Sketch of (a) the commonly used flow cell unit and (b) the micromachined flow cell.

Fabrication of the micromachined flow cell

Design

The use of the optical trapping set-up resulted in several constraints on the micromachined flow cell (see fig. 2).

(i) The flow channel has to be optically transparent for the used wavelengths. The flow channel is etched in silicon and Pyrex is chosen as a cover glass material.

(ii) The cover glass must be matching the objective correction. The objective in the common set-up is corrected for a standard glass coverslip thickness of 170μm with a refractive index of 1.52. With this objective an optical trap is created at a depth in the flow channel of maximum 80μm. The refractive index of Pyrex is 1.47, thus its thickness is about 175μm.

(iii) There is a minimum width for the transparent flow channel. The maximum angle of incidence is about 65° for a beam coming from an objective with NA=1.2, in water. When the trap is at the maximum of 80μm underneath the cover glass, the

cover glass must be optically flat and transparent over a distance of at least 80μm/sin(65) = 340μm. The flat cover glass area is chosen to be 500μmx30mm.

(iv) The distance between the pipette and the cover glass has to be less than the maximum depth of the trap to the cover glass.

(v) A pipette tip opening of 0.5 to 1.0μm diameter is chosen to grip a bead of 2μm. With this size the bead will be on top of the opening and not 'inside' the opening. A round opening would be preferable to avoid leakage along the bead.

(vi) The flow cell should have a small volume. The flow channel is chosen to be 500μm wide, 100μm high, and 40mm long of which 30mm is transparent. The total volume will be not bigger than 2μl.

Micropipettes

In figure 2 the general design of a micromachined flow cell with integrated micropipettes is shown. The fabrication of the flow channel in both Pyrex and silicon is based on bulk wafer processing. The micropipettes are made of silicon nitride based on the buried channel technology (BCT) [14]. A powder blasting process of Pyrex is used to make the connection holes to the "macro world" for the flow channel and the micropipettes. The two wafers are anodically bonded together.

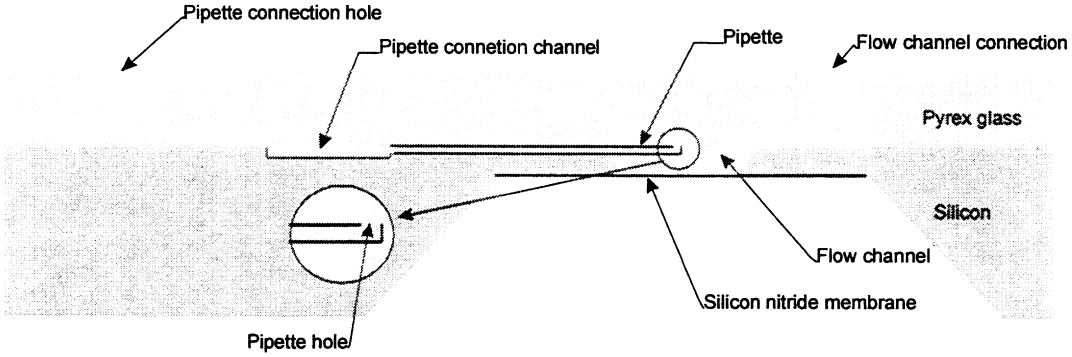


Fig. 2. Cross section sketch of a integrated micropipette in a flow channel.

The outline of the basic steps to create the free standing micropipettes using buried channel technology is given in fig. 3. A 1μm wide trench, that is made in silicon by Reactive Ion Etching (RIE) (step a) is coated with a protection layer (thermal oxide) (step b). The layer is removed from the bottom of the trench by RIE (step c), and the buried structure is made by an isotropic etch of the trench (step d). Then the protection layer is removed and the structure is made by growing in the trench a layer of LPCVD silicon nitride (step e), followed by etching away in KOH the surrounding silicon resulting in a free standing micropipette. (step f). Due to BCT process, the opening of the pipette will be on the top side of the pipette.

The BCT is a single-wafer process, is self-aligned and uses one-sided processing without the need of complicated assembling techniques. Thus, the pipette definition needs a single mask and a single wafer.

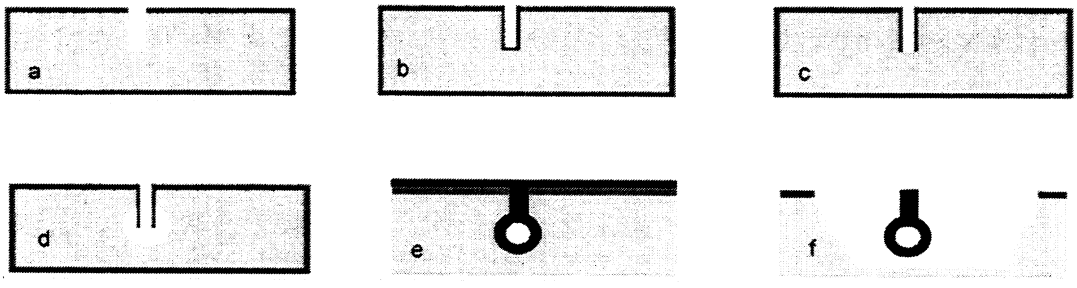


Fig. 3. Schematic principle of the free standing micropipettes using buried channel technology (BCT).

Based on the principle of BCT four different shaped pipettes can be designed (fig. 4).

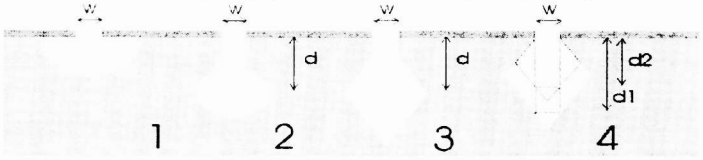
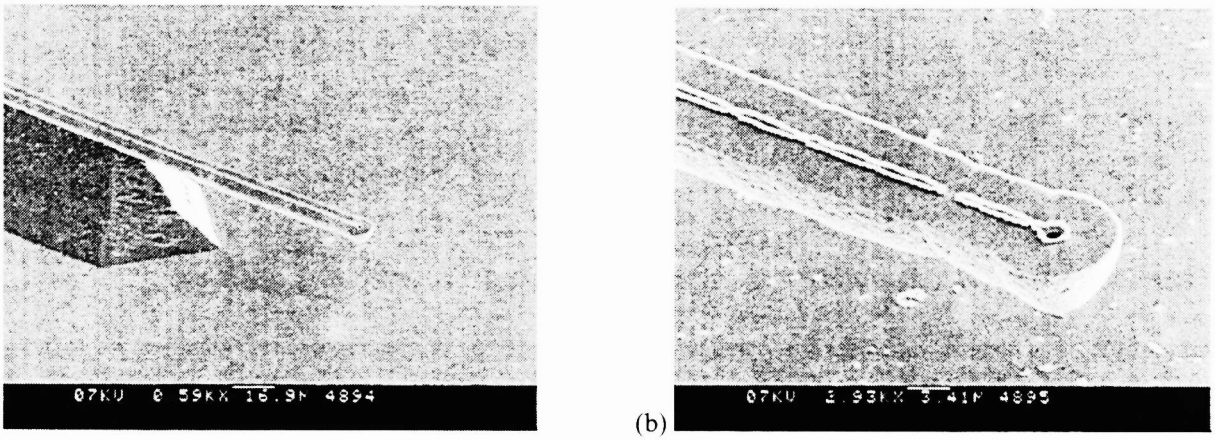


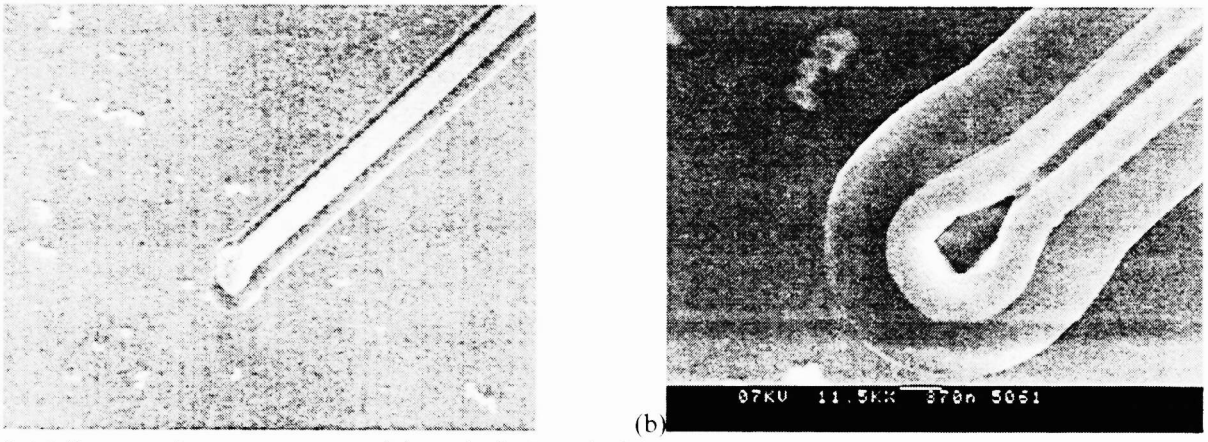
Fig. 4. Four different shapes for pipettes in silicon possible with BCT.

- Shape 1 can be made by an isotropic under etch of a slit etched in a silicon oxide mask. This is the easiest way to make a micropipette. An inconvenience with this type is that when a bead with a DNA molecule is hold on top of the hole, the DNA may stick to the flat top of the pipette (fig. 4-1 and fig. 5). A larger distance between the pipette opening, where the bead is captured, and the pipette surface would be preferable.
- Shape 2 can be achieved by an isotropic under etch of the trench done by RIE (fig. 4-2 and fig. 6) or by a wet etching. A problem with a wet etching process can be the wetting of the trench.
- Shape 3 can be achieved by an anisotropic under etch of the trench done by RIE (fig. 4-3). When a KOH etch solution is used, the walls of the channel will be smooth because of the low etch rate of the [111] planes of the silicon. Disadvantage is that the obtained result depends on the crystal orientation of the silicon.
- Shape 4 uses also a KOH etch solution (fig. 4-4 and fig. 7) and has the advantage that the size of the channel easily can be regulated by the trench depth (d), and there is no need for bottom etching of the trench. Disadvantage is again that the obtained result depends on crystal orientation.



(a) (b)

Fig. 5. (a) Free standing micropipette of shape 1. (b) Detail of the pipette tip showing the hole with a diameter of 1 μm.



(a) (b)

Fig. 6. (a) Free standing micropipette of shape 2. (b) Detail of the pipette tip (top view).

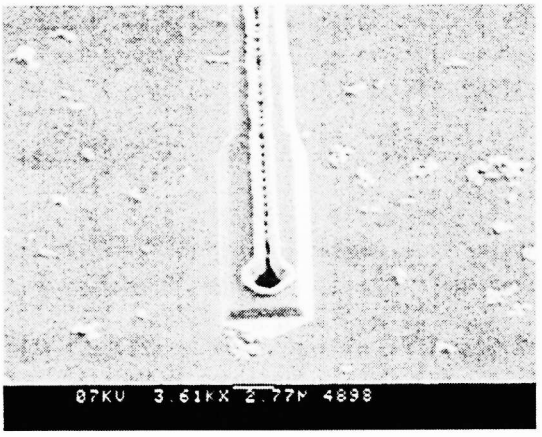
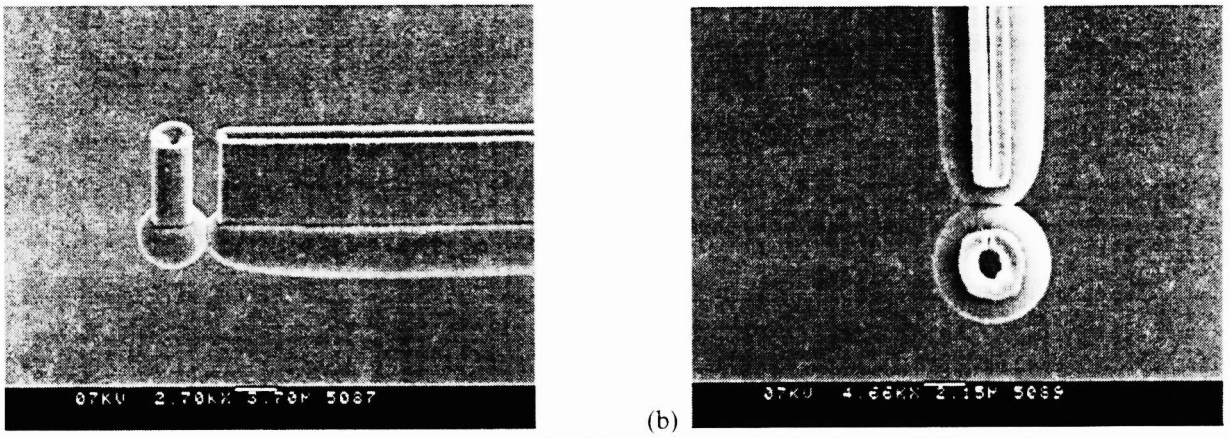


Fig. 7 Micropipette of shape 4.



(a) (b)

Fig.8 Micropipette of shape 2 to achieve a more circular-like opening. (a) side view, and (b) top view.

As can be seen in fig. 6 and fig. 7, the circular opening of the pipette is deformed due to the influence of the trench. For a better opening definition a different shape was designed, fig. 8 [18].

In shape 2 and 3 the depth of the structure underneath the surface can be tuned by the depth(d) of the trench (fig. 3a). The trench depth is limited by the width(W) of the trench and by the maximum aspect ratio that can be achieved during RIE etching [19]. The other limit to the trench depth is the easiness in removing the trench bottom [14].

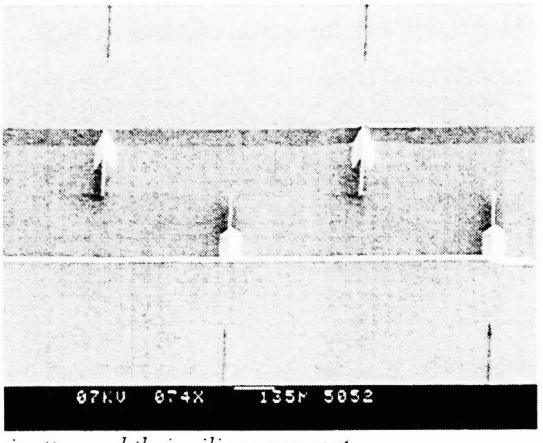


Fig. 9. SEM picture of several micropipettes and their silicon support.

Many micropipettes are simultaneously integrated in a flow channel of 100 μm height, 500 μm wide and 3 cm long (fig. 9). This small channel volume, 2 μl, requires only very small sample volume. The micropipettes shown have a length of 130 μm, width of 5-10 μm, and a circular shape opening of 1μm.

Flow channel

From fig. 2 it can be seen that the micropipettes connections and the upper part of the flow channel are made in a Pyrex glass wafer. The Pyrex wafer is patterned and etched in 50% HF solution using a 1μm sputtered silicon layer as mask.

The next step is the powder blasting through the Pyrex wafer creating a 300 μm in diameter connection holes [15]. For this, a layer of dry film resist (Ordyl BF405) is applied on the other side of the Pyrex wafer (not the structured side) and patterned with photolithography. Then, the Ordyl is removed, the Pyrex wafer is diced, the sputtered Si mask is removed by KOH and the flow channel side is anodically bonded to the silicon wafer in order to assemble the flow cell.

The fluidic connections between the flow cell and a holder uses a new packing material instead of a macroscopic connection (like a Viton O-ring causing a large dead volume). This packing material is, again, the dry film resist (Ordyl BF405), which prevents leakage and simplifies the interfacing with the "macro world" [18].

Results and Discussions

Tests, performed with shape 2 micropipettes, have shown that the pipettes are open (Fig.10). As can be seen the micropipettes can be used to locally inject small air bubbles into a fluid flow.

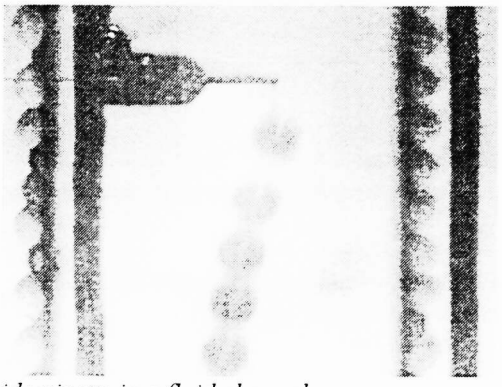


Fig. 10. Making air bubbles with a nitride pipette in a fluid channel.

From the first experiments with the polystyrene beads and micropipettes in a flow channel it could be seen that the beads were sticking to the silicon nitride. Fig. 11 shows a lot of beads sticking to the micropipette. The strength of the sticking force is such that the beads could not be released with the optical tweezers (~70pN).

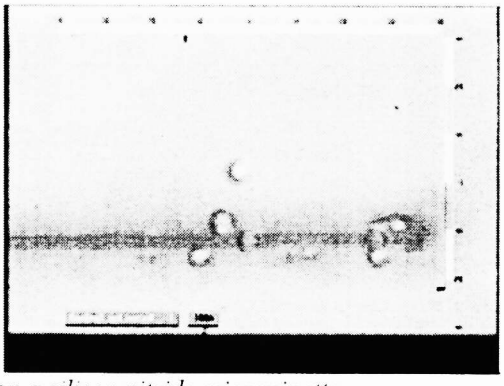


Fig. 11. Sticking of polystyrene beads on a silicon nitride micropipette.

The beads on the pipette could easily be removed when the flow cell was washed with 1% SDS (sodium dodecyl sulfate) solution or with BSA (bovine serum albumin). Figure 12a shows a micropipette and a bead that is gripped by the optical tweezers. Then, the bead is transferred to the pipette opening and gripped, fig. 12b.

In fig. 13a, the DNA molecule is held in between the beads, and the force sensor marker shows zero force. Of course, the DNA itself can not be seen using optical wavelength due to its extreme small diameter (2nm). By moving the pipette away from the optical trap the DNA molecule is stretched, fig. 13b.

The force-extension curve obtained with the micromachined flow cell and micropipettes, fig. 14, shows a similar behaviour as the typical one (not shown) [13]. In the graph there are two curves. The diamond points represent the stretching of the DNA, and the square points are measured during the relaxation of the same molecule.

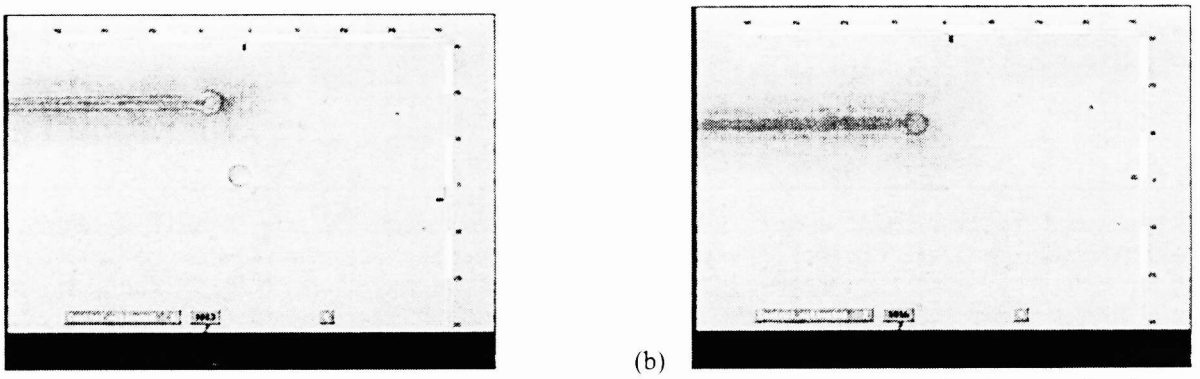


Fig. 12. (a) A 2μm polystyrene bead hold by the optical tweezers next to the micropipette. (b) the same bead gripped by the micropipette

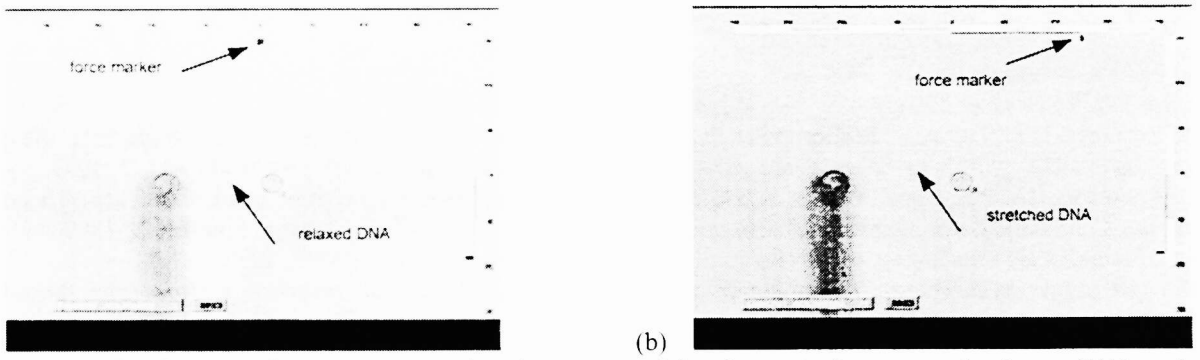


Fig. 13. Two beads gripped on a micromachined pipette and by the optical tweezers having a DNA molecule (not observable) hold in between; (a) no force applied;(b) 30pN force applied.

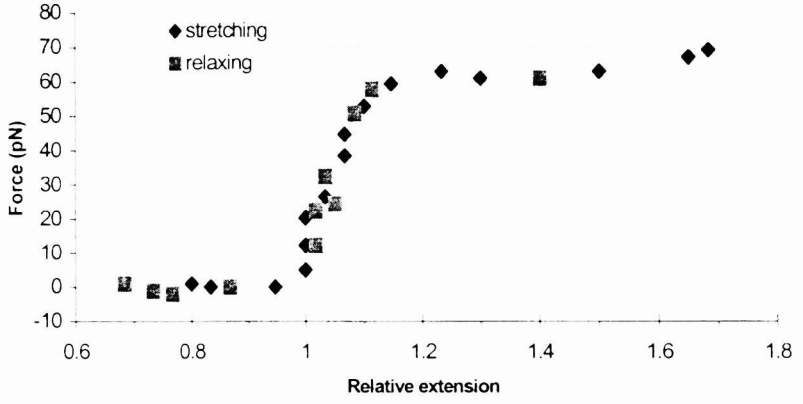


Fig.14. Experimental stretch measurements with the micromachined flow cell and the micropipettes.

The behaviour of the single molecule up to a few pN is the entropic elasticity as described by [11]. As soon the contour length of DNA is reached (relative extension L/L_0 =1) the force increases very fast up to about 60pN. For L/L_0 between 1 and 1.1 the DNA shows a spring-like behaviour. Above this level the DNA molecule is stretching out within a force of only a few pN.

An effect was observed when the optical trap was brought within 5μm from the pipette: the light from the laser was deflected by the pipette. The light of the laser beam diverges rapidly below the bead given an incorrect force measurement.

The present micropipettes (ca. 12μm high, 5-10μm width) are suitable for the study of the mechanical properties of long DNA molecules (> 10μm).

For chemical study of DNA, shorter DNA molecule (5μm) has to be used. For this investigation, the micropipettes have to be downscaled. A smaller pipette (outer diameter 2-3μm) can be constructed by etching a less deep trench with a smaller width (<1μm) and reducing the etch time of the isotropic etch (fig. 3d).

Conclusions

We have demonstrated the possibility to integrate micropipettes into a flow channel to study mechanical and structural properties of DNA molecules. Moreover, various shapes of the micropipettes have been fabricated having the convenience of choosing for each type of experiment the best pipette shape and size. The conventional flow cell with glass capillary pipettes have been replaced successfully by this micromachined flow cell. Force-extension curves from both the conventional and micromachined technology were found to be in excellent agreement. For chemical study of DNA, the micropipette should be downscaled.

References

[1] *Science* **283**, 12 March 1999, p.1668-1695.

[2] *Science* **282**, 16 October 1998, p.429, 484-487, 433b, 682-689, 1046-148.

[3] R.G. Sosnowski, E. Tu, W.F. Buttler, J.P. O'Connell, M.J. Heller, Rapid determination of single base mismatch mutations in DNA hybrids by direct electric field control, *Proc. Natl. Acad. Sci. USA* **94**, p.1119-1123, 1997.

[4] R.C. Anderson, G.J. Bogdan, Z. Barniv, T.D. Dawes, J. Winkler, K. Roy, Microfluidic biochemical analysis system, *Proc. Int. Conf. Solid-State Sensors and Actuators, Transducers'97*, p. 477-480, Chicago, June 16-19, 1997.

[5] S.P.A. Fodor, DNA-sequencing: massively parallel genomics, *Science* **277**, p.393-395, 1997.

[6] C.H. Mastrangelo, M.A. Burns, D.T. Burke, Microfabricated devices for genetic diagnostics, *Proc. IEEE* **86**, p.1769-1786, 1998.

[7] *Science* **284**, 11 June 1999, p.1754.

[8] R.H. Carlson, C.V. Gabel, S.S. Chan, R.H. Austin, Self-sorting of white blood cells in a lattice, *Phys. Rev. Lett.* **79**, p. 2149, 1997.

[9] S.W. Turner, A.M. Perez, A. Lopez, H.G. Craighead, Monolithic nanofluid sieving structures for DNA manipulation, *J. Vac. Sci. Technol.* **B 16**, p.3835-3840, 1998.

[10] M.E. Fauver et al., Microfabricated cantilevers for measurement of subcellular and molecular forces, *IEEE Trans. Biomed. Eng.* **45**, 1998.

[11] S.B. Smith, Y. Cui, C. Bustamante, Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules, *Science* **271**, p.795-799, 1999.

[12] P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J. Viovy, D. Chatenay, F. Caron, DNA: an extensible molecule, *Science* **271**, p.792-794, 1999.

[13] M.L. Bennink, O.D. Schärer, R. Kanaar, K. Sakata-Sogawa, J.M. Schins, J.S. Kanger, B.G. de Grooth, J. Greve, Single molecule manipulation of double-stranded DNA using optical tweezers, *Cytometry* **36**, p. 200-208, 1999.

[14] M.J. de Boer, H.V. Jansen, W. Tjerkstra, M.C. Elwenspoek, Micromachining of Buried Micro Channels in silicon, accepted for *JMEMS*, March 2000.

[15] H. Wensink, J.W. Berenschot, H.V. Jansen, M.C. Elwenspoek, Micromachining by powder blasting, accepted to *IEEE International Micro Electro Mechanical Systems Conference, MEMS 2000*, 23-27 January 2000, Miyazaki, Japan.

[16] A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu, Observation of a single-beam gradient force optical trap for dielectric particles, *Optics Letters* **11**(5), p.288-290, 1986.

[17] L.P. Ghislain, N.A. Switz, W.W. Webb, Measurement of small forces using an optical trap, *Rev. Sci. Instrum.* **65**(9), p.2762-2767, 1994.

[18] C. Rusu, R. van't Oever, M. de Boer, H.V. Jansen, E. Berenschot, M.L. Bennink, J.S. Kanger, B.G. de Grooth, M.C. Elwenspoek, J. Greve, A. van den Berg, J. Brugger, Fabrication of micromachined pipettes in a flow channel for single molecule handling of DNA, accepted to *IEEE International Micro Electro Mechanical Systems Conference, MEMS 2000*, 23-27 January 2000, Miyazaki, Japan.

[19] H.V. Jansen and M.C. Elwenspoek, *Silicon Micromachining*, University Press, 1998.