

# AN OPEN MICROFLUIDIC DEVICE WITH ACTIVE VALVES FOR ACCURATE TRAPPING OF DNA BY SILICON NANOTWEEZERS

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## ABSTRACT

This paper demonstrates the real-time monitoring of  $\lambda$ -DNA molecule trapping by silicon nanotweezers in an open microfluidic chamber. An active microfluidic device has been developed aiming to allow the insertion of MEMS tweezers and to control the biological solution inlets for an accurate sensing of bioreactions through the tweezer mechanical frequency response.

**KEYWORDS:** Nanotweezers, DNA trapping, Open microfluidic, Active valves

## INTRODUCTION

During the past years, direct manipulation of DNA molecules has expanded our understanding on molecular biology. New tools for basic investigations on DNA mechanical properties have enabled single-molecule assays of enzyme mechanisms, clearing the nature of interactions between DNA and proteins and the forces within which the cellular machinery operates [1-4]. Hence a huge interest exists on systematic and real-time molecular analysis, we proposed a micromechanical-electro system (MEMS) for the manipulation of biomolecules and the characterization of interactions between DNA and enzymes [5,6].

As the characterization of biological phenomena with MEMS tweezers is based on parameter tracking, obtaining reliable biological environment for high sensitivity experiment presents a real challenge for relevant experiments. In this new development, a complementary microfluidic device has been designed and fabricated in order to achieve control of the solution inlet/outlet sequences and volumes (Figure 1). Here, we performed biological experiments in reliable conditions settling reaction time and preventing evaporation.

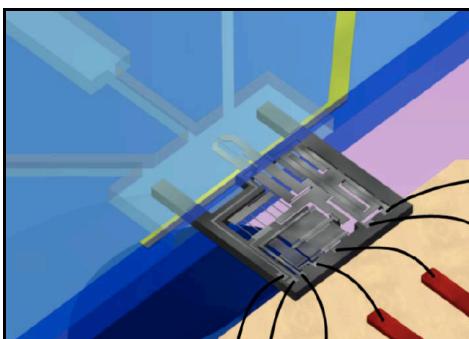


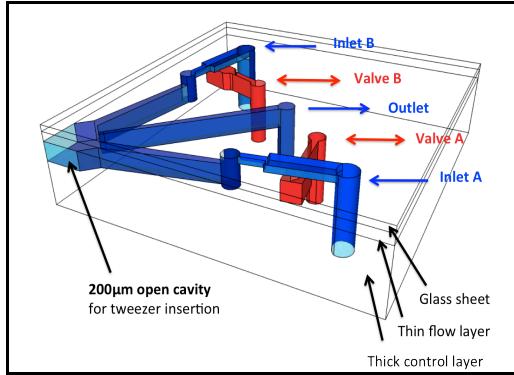
Figure 1: 3-D view of the experimental setup combining nanotweezers and a specific microfluidic device

## METHODS

The MEMS tweezers consist of a pair of opposing nanotips. The distance between the tips can be adjusted with nanometer accuracy by a comb drive actuator and measured by a capacitive sensor. With tweezers, bundles of DNA can be repeatedly trapped between their tips in a droplet, and the temporal development of bioreactions on  $\lambda$ -DNA bundle was measured with HindIII restriction enzymes [6]. On the other hand, as passive microfluidic devices do not allow a proper filling of an open chamber and remote control microfluidic system cannot provide enough quick response to compensate evaporation, techniques of soft lithography have been used to make monolithic valves from polydimethylsiloxane (PDMS) [7].

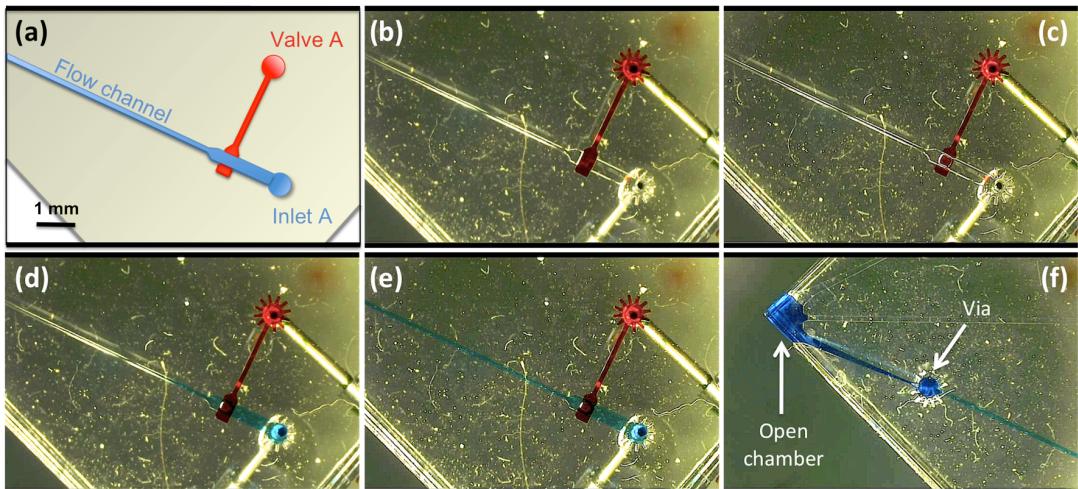
We fabricated our valves using crossed-channel architecture. The device is fabricated by replica molding from two masters and sealing the layers together. A thin layer is produced for controllable flow channels when a thick layer is produced for the control channel and the implementation of an open reaction chamber. The valve membranes are formed where the control and the flow channels intersect orthogonally. The control layer is bonded underneath the flow layer forming push-up valves, and the flow layer is sealed with a glass slide as top layer for optical convenience (Figure 2).

Flow channel wafer is patterned with AZ-4903 photoresist and the resist is reflowed by thermal heating to form rounded-shape. The shape of the flow channel is consequential for proper actuation and hermetic closing of the valve. The 10- $\mu\text{m}$  thin elastomeric membrane is created above the patterns by adjusting the PDMS spin coating speed. Control channel wafer is patterned with SU-8 photoresist. Lastly a 200- $\mu\text{m}$  chamber is implemented to insert the 25- $\mu\text{m}$ -thick of tweezer probes.



*Figure 2: 3-D schematic of the open microfluidic device: Flow channels and the open reaction chamber (in blue) and control channels (in red).*

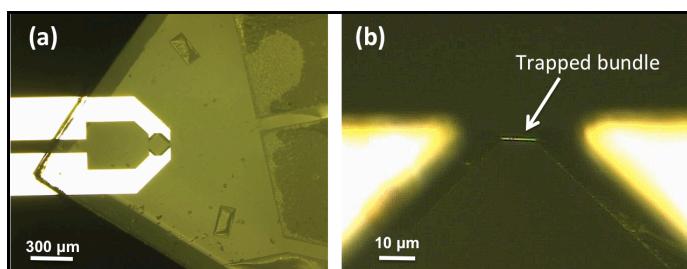
At the cross-section, channels are 600- $\mu\text{m}$  wide, making the active area 600  $\mu\text{m}$  by 600  $\mu\text{m}$  and determining the valve actuation pressure (100 kPa). When pressure is applied to the lower channel, the membrane deflects upward and closes the upper channel stopping the flow (Figure 3-b). Via was implemented to allow liquid transitions between the flow channels and the open chamber (Figure 3-f). Finally, combined to a convenient solution pressure (<10 kPa), the response time of the device is fast enough to precisely fill the reaction chamber making it suitable for the control of the biological solutions.



*Figure 3: Video sequences of the controlled filling of the reaction cavity. (a) Red channel is the membrane-valve control while blue channel is the biological solution channel. (b) Red control channel is under pressure (100 kPa) closing the valve. (c) Control pressure is released; the valve is open. (d) The pressure released; blue “biological” solution crossed the valve. (e) The blue “biological” solution is reaching the reaction cavity. (f) The blue “biological” solution is properly filling the open reaction cavity.*

## EXPERIMENTAL

After have properly filled the reaction chamber, the probes of tweezers are introduced into from the open side (Figure 4a). An AC voltage (1 MHz, 20 Vpp) is applied between the tips, as by dielectrophoresis, DNA molecules elongate along the most intensive line of the resulting electric field [5]. During the trapping, the frequency response of the tweezers is continuously recorded following the phase rotation at the resonance frequency. At the end, a microscopic visualization confirmed that a DNA bundle is trapped between the two tips (Figure 4b).



*Figure 4: Tweezers in experience: (a) tweezers inserted in the open reaction chamber, (b) a trapped  $\lambda$ -DNA bundle bridges tweezer gap.*

## RESULTS AND DISCUSSION

As trapping proceeds,  $F$  increases due to the addition of DNA bundle stiffness  $k_{bundle}$  (Equation 1, Figure 5). At the same time,  $Q$  tends to decrease as the viscous losses  $\nu_{bundle}$  increases with the bundle formation (Equation 2, Figure 5). Precise frequency measurements allow the sensing of  $5.10^{-3}$  Hz shift, around the typical resonance frequency (2.5 kHz), corresponding to 10  $\lambda$ -DNA molecules stiffness. From the equations 1 and 2 and knowing the single molecule rigidity ( $k_{\lambda\text{-DNA}}=3.10^{-5}$  N/m, [1]), the time evolutions of the bundle rigidity and viscosity can be deduced (Figure 6). Focusing on the first 300 seconds, the trapping rate was 0.9 molecule/second.

$$F = \frac{1}{2\pi} \sqrt{\frac{k_{TW} + k_{bundle}(t)}{M_{TW}}} \quad \dots \dots \dots \quad (1)$$

$$Q = \frac{\sqrt{(k_{TW} + k_{bundle}(t))M_{TW}}}{\nu_{TW} + \nu_{bundle}(t)} \quad \dots \dots \dots \quad (2)$$

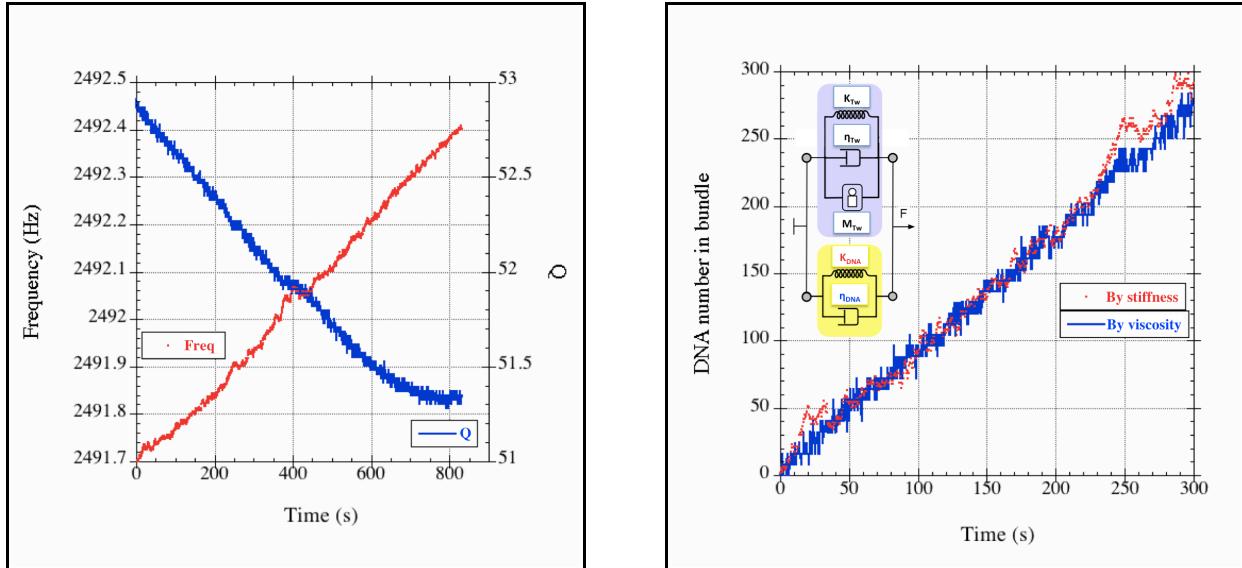


Figure 5: (On left) Tweezers +  $\lambda$ -DNA bundle resonance frequency and quality factor vs. DEP time.

Figure 6: (On right) Evolution of trapped  $\lambda$ -DNA molecules vs. DEP time. Number of trapped molecules is deduced from bundle rheological model and single molecule rigidity ( $k_{\lambda\text{-DNA}}=3.10^{-5}$  N/m [1]).

## CONCLUSION

The sensing of DNA molecule trapping with MEMS tweezers was demonstrated with high sensitivity. An active microfluidic device allowed dynamic control of the biological solution inlets. Moreover in order to sense slow enzyme kinetics with HindIII (until 1 hour), the device makes possible the compensation of drawbacks resulting from the solution evaporation. This new method shows the possibility to control the experiment conditions for accurate and systematic biological tests on filamentary molecules with the MEMS tool and electronic read-out.

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