

# FAST SEPARATION OF $\lambda$ - AND T2-DNA WITH ELECTRODELESS DIELECTROPHORESIS

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## ABSTRACT

The fast separation of long DNA via electrodeless dielectrophoresis in a simple and easy-to-fabricate microfluidic device is presented. An AC voltage is applied to an array of periodically arranged posts creating dielectrophoretic traps. Size-dependent migration velocities are obtained by applying an additional DC voltage, which drives the DNA through the structured microchannel. With this technique we are able to achieve fast and efficient separations of  $\lambda$ - and T2-DNA. The mechanism is analysed in terms of a Kramer's rate and a notable change in the diffusion coefficient of  $\lambda$ -DNA is observed.

**Keywords:** DNA, separation, dielectrophoresis, microfluidics

## 1. Introduction

The fast and reliable separation of large DNA molecules is still a demanding problem in separation science. The established method of slab gel electrophoresis with pulsed electric fields is very time consuming (10h up to 200h). Capillary electrophoresis with diluted gels represents a fast alternative, however with similar drawbacks in size limitation.

Harnessing the exerted forces of nonuniform AC electric fields onto polarizable objects is well established. By extending this method using a targeted combination of microstructures and electric fields, novel techniques for the separation of biomolecules can be developed. The ion cloud around DNA in aqueous solution renders it polarizable, thus a dipole can be induced enabling the interaction with inhomogeneous electric fields. Although the mechanism of DNA polarization is not yet understood in detail [1], it was experimentally demonstrated that DNA exhibits positive dielectrophoresis. The necessary nonuniform electric field can be designed with non-conducting posts and the DNA is trapped at the locations of the highest field gradient in between two posts known as electrodeless dielectrophoresis of DNA [2]. Ajdari and Prost made in 1991 a theoretical proposal to exploit dielectrophoresis for DNA separation [3]. In this paper, we extend these previous studies and present the first separation of  $\lambda$ - and T2-DNA in a microfluidic chip via electrodeless dielectrophoresis.

## 2. Methods

The monolithic separation chip is made of poly(dimethylsiloxane) (PDMS) based on soft lithography. Briefly, contact lithography of SU-8 on a Si-wafer is followed by casting PDMS from the SU-8 masterwafer. The cured and structured PDMS is peeled

off and, after an oxygen plasma treatment, it is covered with a glass slide which was previously spincoated with PDMS. Fig. 1 shows an electron micrograph of the injector (1) and the first two rows of the array of posts, which extends over 3.8mm in the separation channel (4). The channels are 95 $\mu\text{m}$  wide, the posts are 7.4 by 2.2 $\mu\text{m}$  with a spacing of 2.3 $\mu\text{m}$  and a row distance of 21.1 $\mu\text{m}$ . The DNA is dissolved in 10mM phosphate buffer containing 2mM NaCl, 1mM EDTA, 0.1% (v/v) POP-6, 2 $\mu\text{l/ml}$   $\beta$ -mercaptoethanol and YOYO-1 for fluorescent staining and is injected via a cross injector. For the separation of  $\lambda$ -DNA (17.5pM) and T2-DNA (6pM), a sample plug is driven through the separation channel by a DC voltage ( $U_{\text{DC}}$ ) of 12V applied between the electrodes in the reservoirs (3) and (4) with a distance of 7mm. An AC voltage ( $U_{\text{AC}}$ ) of 60Hz, also applied at the electrodes (3) and (4), is increased incrementally from 150V to 189V, with steps of 0.6V every 3 seconds. When the final value is reached, the DC voltage is switched off, and a steady state electropherogram is recorded by scanning the fluorescence signal of the separated and trapped DNA zones in the structured channel.

Video sequences at 10 fps with single observable  $\lambda$ -DNA molecules are recorded for varying AC voltages at 60Hz with  $U_{\text{DC}} = 12\text{V}$  and analysed in terms of trapping and migration times.

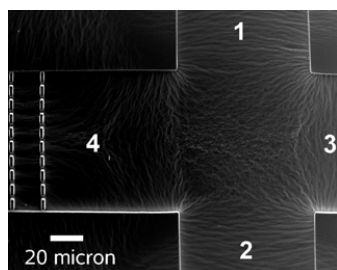


Figure 1. SEM image of a section of the microfluidic chip.

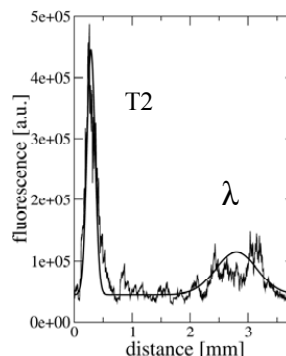


Figure 2. Electropherogram of  $\lambda$ - and T2-DNA with gaussian fits as a guide to the eye [4].

### 3. Results and Discussion

The successful separation of  $\lambda$ (48.5kbp)- and T2(164kbp)-DNA is shown in fig. 2 [4], with a separation time of only 200s. The T2-DNA is trapped at the beginning of the separation channel, whereas the  $\lambda$ -DNA molecules are only captured at a distance of 2.8mm from the injector. The sample is separated with baseline resolution.

The mechanism of the separation can be elucidated by comparing the Kramer's rate, i.e. the inverse time required to escape from a trap and to migrate to the next. Figure 3 shows the Kramer's rate of the two sorts of DNA versus  $U_{\text{AC}}$ . Above amplitudes of 125V the rates for the two DNA species differ significantly, clearly justifying the applied  $U_{\text{AC}}$  values in the separation experiment. One can conclude that the separation is

based on the faster escape of  $\lambda$ -DNA out of the trap. This can be due to different polarizabilities and to different diffusion coefficients of the two DNA species [3]. In order to investigate the unusual broadening of the  $\lambda$ -DNA peak in fig. 2, we determined the dispersion of the  $\lambda$ -DNA according to [5] (see fig. 4). With increasing trapping voltages, i.e. above 140V, the diffusion coefficient is significantly increased. This corresponds to longer trapping times (fig. 3) and explains the dispersion of the  $\lambda$ -DNA peak in fig. 2. The origin of this unusual increase is currently under experimental investigation.

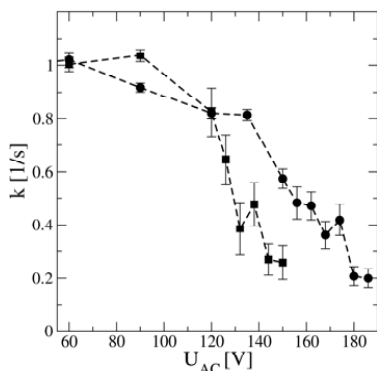


Figure 3. Kramer's rate  $k$  versus  $U_{AC}$ .

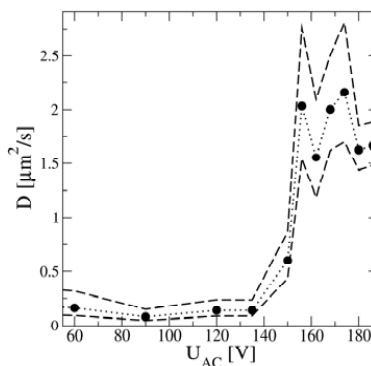


Figure 4. Diffusion coefficient of  $\lambda$ -DNA versus  $U_{AC}$  (dashed lines indicate the error).

#### 4. Conclusion

Our new microfluidic method exploiting electrodeless dielectrophoresis enables the efficient separation of long DNA strands within only 200s. The mechanism of the separation can be understood in terms of a size dependent trapping of DNA manifested by the observed Kramer's rate for the escape out of the dielectrophoretic traps. We expect that this new separation method for long DNA molecules can selectively be adapted and optimized by tuning the electric driving parameters (AC and DC) to address an even broader size range in the future [4].

#### 5. References

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