Nanofluidic system for matrix-free DNA analysis

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Abstract

Chromosomes are the universal support of the genomic material, and their analysis at the genome level is among the most active field of research and innovation. Most analytical tools have the ability to sequence short DNA fragments, which are subsequently registered by computers to define a complete genome. This cost-ineffective situation is mostly related to the history of molecular biology techniques, which have been developed to characterize DNA chains of less than ~20 kbp. We posit that micro- and nano-technologies offer new solutions to manipulate long DNA fragments, and hold great promise to the analysis of large scale genomic rearrangements which are common in cancer **[1]**.

In this report, we describe a novel technology to separate biomolecules by size, which does not require the use of a separation matrix. The system consists of a silicon fluidic chip obtained by conventional photolithography, followed by dry etching (Fig.1.a). The slit-like geometry enables to monitor the environment of biomolecules, and we use a combination of hydrodynamic and electrophoretic to convey biomolecules (Fig.1.b), so that three control parameters, namely the geometry, the pressure, and the electric field, enable to control the progression of biomolecules in the channels. The application of an hydrodynamic field alone does not induce separation, whereas the application of an electric field acting opposite to the hydrodynamic flow improves the performances of our system: the chromatogram obtained with 500 mbar and 30 V shows sharp peaks for ten DNA species spanning 500 to 10000 bp in less than 8 minutes (Fig. 2). Small DNA molecules migrate faster than large ones, as observed with conventional gel electrophoresis but not with hydrodynamic separations [2]. Using real time single molecule nanoscale imaging, we show that size-dependent migration is achieved through transverse displacements: molecules accumulate along streamlines according to their molecular weight, so that their progression along channels is determined by their size. This phenomenon is enhanced with the geometry of the channel and with the rheological properties of the fluid, and we show the successful separation of DNA molecules from 100 bp to 100000 bp in minutes, a performance inaccessible to most technologies available on the market. Most importantly we demonstrate sharp variations of DNA velocity as a function of its size characterized by power-law scalings up to ~-2,75 (Fig. 3), thus outperforming conventional electrophoresis that is associated to a power-law scaling of -1 [3].

Overall this study is a demonstration of the potential of fluidic technologies for the life sciences. Our technology is readily adapted for integration in Lab-on-Chip applications, and it is well-suited to perform analytical operations on whole-chromosomes for novel biodiagnostic applications.

References

[1] Stephens P.J. *et al*., *Cell*, **144** (2011) 27-40.

[2] Liu K.J *et al.*, *Journal of the American Chemical Society*, **133** (2011) 6898-6901

[3] Viovy J.L., *Review of Modern Physics*, **72** (2000) 813-872

Figures

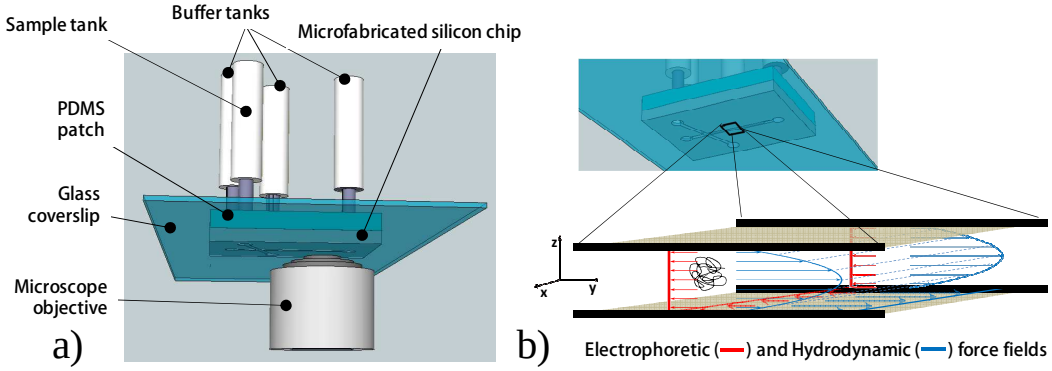

Figure 1 Description of the integrated fluidic system and the actuation technology. a) Sketch of the chip on a coverslip observed on an inverted microscope. All fluidic tanks are pressure and voltage controlled. b) Description of the actuation modes: red arrows show the electrophoretic field and the blue line stands for the Poiseuille flow.

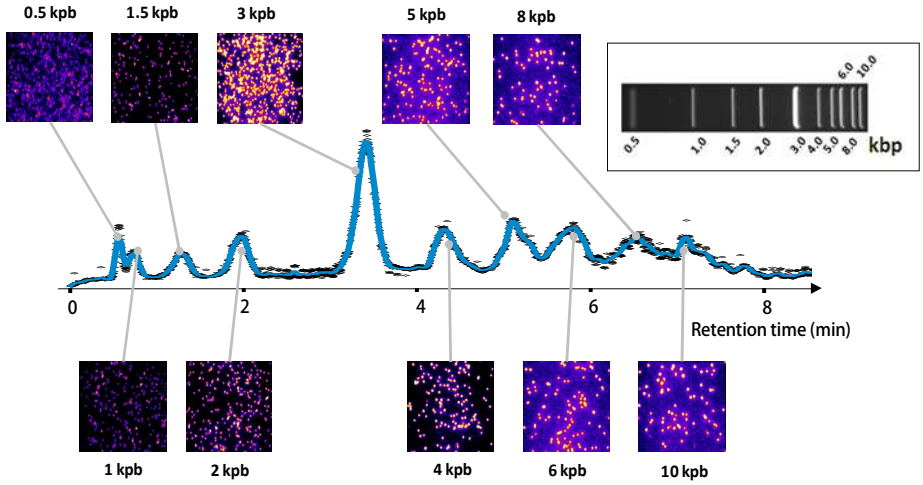

Figure 2 Chromatograms obtained for a commercial kb ladder @ [500 mbar; 30 V] demonstrating clear resolution of the 10 DNA strands of the sample in less than 8 minutes. Associated to each peaks are video frames obtained on the EMCCD camera, allowing for size discrimination based on single molecule intensity. The inset on the right is a photograph of the ladder after migration in an agarose gel.

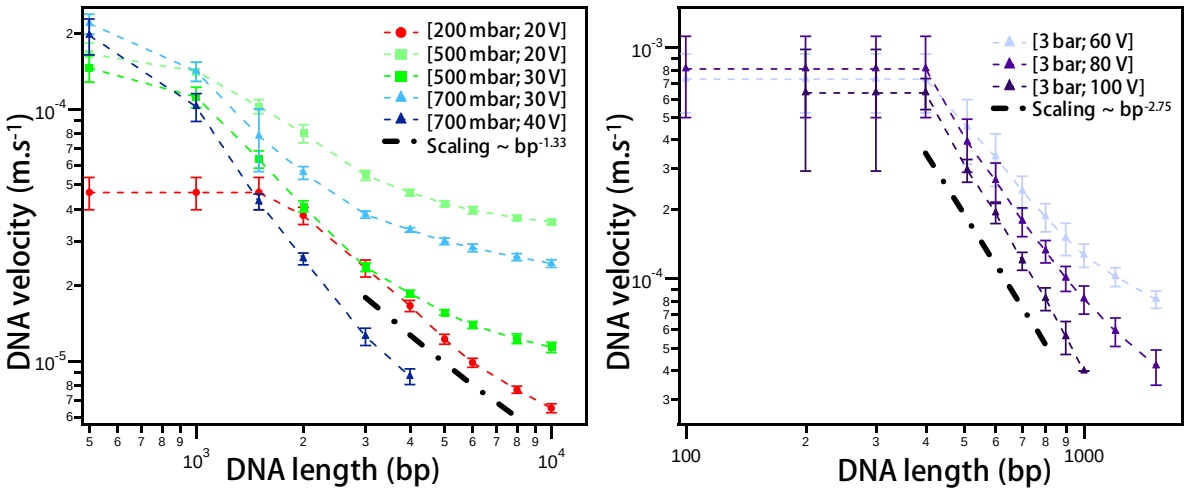

Figure 3 Velocity of DNA molecules as function of their lengths. kb ladder (left). 100 bp ladder (right). Pressure drops and voltages are indicated in inset. Tuning of the electric field and pressure drop induces sharp variations in mobility with the molecular weight. The highest variations are associated to a power scaling of ~ -1.33 and ~ -2.75 (black dashed and dotted lines).