

Sub-base-pair resolution during DNA separation in an optofluidic chip

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Introduction. DNA sequencing in a lab-on-a-chip aims at providing cheap, high-speed analysis of low reagent volumes to, e.g., identify genomic deletions or insertions associated with genetic illnesses. Detecting single base-pair insertions or deletions from DNA fragments in the diagnostically relevant range of 150–1000 base-pairs requires a sizing accuracy of $S < 10^{-3}$, while only $S < 10^{-2}$ were reported [1]. Here we demonstrate a sizing accuracy of $S = 4 \times 10^{-4}$, thereby paving the way for the envisaged applications.

Experimental. A microfluidic channel network and reservoirs were patterned photolithographically and wet-etched into fused silica glass and sealed off by bonding a piece of fused silica glass on top [2], see Fig. 1. An optical waveguide was post-processed by femtosecond-laser writing [3]. The inner walls of the microfluidic channels were coated with an epoxy-poly-(dimethylacrylamide) (EPDMA)-based polymer [4]. Subsequently, the channels were filled with a sieving gel matrix consisting of hydroxyethyl-cellulose, dissolved in His buffer. Two sets of DNA molecules were permanently end-labeled with different dyes to identify their origin [5]. The 12 blue-labeled and 23 red-labeled DNA fragments were separated in size by capillary electrophoresis [1,5], each set excited exclusively by either of two lasers power-modulated at different frequencies and launched through the optical waveguide, their fluorescence detected by a sensitive photomultiplier, and blue and red signals distinguished by Fourier analysis [5]. Results are shown in Fig. 2.

Data analysis. Different calibration strategies for the dependence of migration time on base-pair size were tested: a) use either set of DNA molecules as reference to calibrate the set-up and identify the base-pair sizes of the other set in the same flow experiment, thereby eliminating variations in temperature, wall-coating and sieving-gel conditions, and actuation voltages; b) use the same molecular set as reference and sample (in a real-life experiment the reference set would be the healthy counter-part of an unknown, potentially malign sample set) with the same fluorescence label, flown in consecutive experiments; c) perform cross-experiments based on different molecular sets with different labels, flown in consecutive experiments; also d) self-calibration in the same experiment was analysed. Results are shown in Fig. 3.

Discussion. From the experimental results displayed in Fig. 2 and their analysis shown in Fig. 3 we conclude the following:

- 1) Applying quadratic instead of the usual linear fit functions improves the accuracy of calibration.
- 2) Blue-labeled molecules, see Fig. 2(a), are separated with higher accuracy than red-labeled molecules, see Fig. 2(b), hence different dye labels influence the DNA flow differently.
- 3) Different dye labels affect the formation and microfluidic flow of individual DNA plugs more severely than variations in temperature, wall-coating and sieving-gel conditions, and actuation voltages between consecutive experiments.
- 4) Choosing a single, suitable dye label, combined with reference calibration and sample investigation in consecutive experiments, see the left-hand side of the dark-blue curve in Fig. 3, results in a sizing accuracy of $S = 4 \times 10^{-4}$, enabling detection of single base-pair insertion/deletion in a lab-on-a-chip.

Conclusions. Careful preparation of an optofluidic chip and a suitable calibration strategy results in capillary electrophoretic separation of fluorescent-labeled DNA molecules in the 150–1000 base-pair range with sub-base-pair resolution, thereby enabling detection of single base-pair insertion/deletion in a lab-on-a-chip with low reagent volumes in a few-minute experiment.

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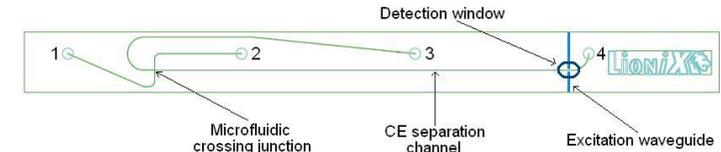
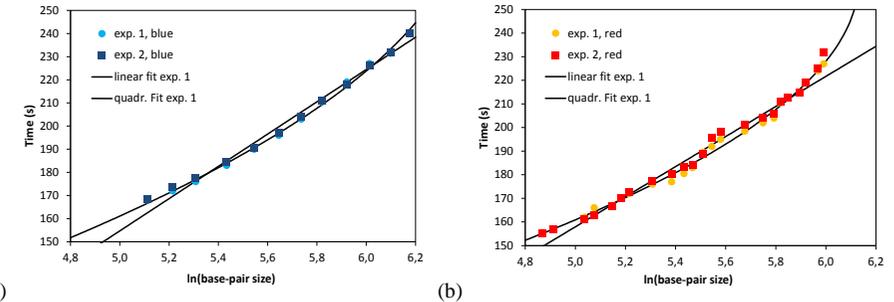


Figure 1. Schematic of optofluidic chip.



(a) (b) Figure 2. Migration time (linear scale) vs. DNA base-pair size (logarithmic scale) of (a) 12 blue-labeled and (b) 23 red-labeled DNA molecules simultaneously migrated and separated in experiment 1 (circles) and experiment 2 (squares).

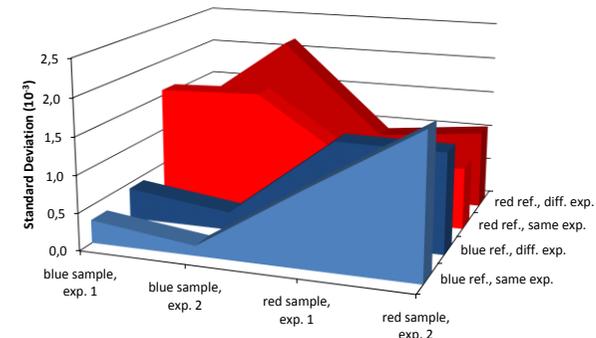


Figure 4. Standard deviation of measured data from quadratic fit function.