Sub-base-pair Resolution by Electrophoretic Separation and Fluorescent Detection of DNA Molecules in an Optofluidic Chip

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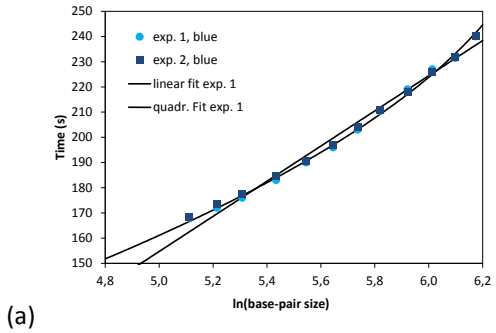
Highlights

Applying capillary electrophoresis, we separate and detect two sets of fluorescent-labeled DNA molecules in the 150–1000 base-pair range and achieve a sizing accuracy of 4×10^{-4}, enabling micro-chip analysis of single-base-pair deletion/insertion.

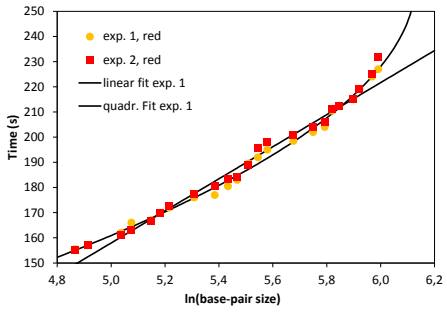
Abstract

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 4×10^{-4}, thereby paving the way for the envisaged applications.

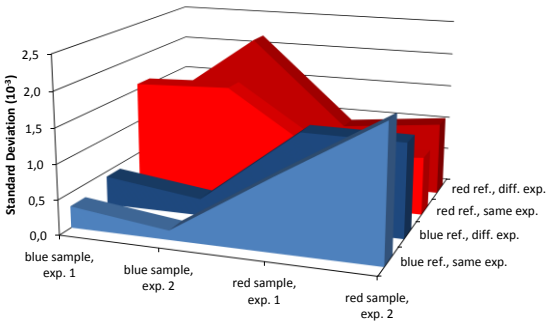
A microfluidic chip (LioniX BV, Fig. 1d) was post-processed by femtosecond-laser writing of an optical waveguide [2]. Two sets of DNA molecules were permanently end-labeled with different dyes to identify their origin [3]. The 12 blue-labeled (Alexa fluor 488) and 23 red-labeled (Alexa fluor 647) DNA fragments were separated in size by capillary electrophoresis, each set excited exclusively by either of two lasers power-modulated at different frequencies, their fluorescence detected by a photomultiplier, and blue and red signals distinguished by Fourier analysis. Different calibration strategies 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 analyzed. From the results (Figs. 1a–b) we conclude: 1) Applying quadratic instead of the usual linear fit functions improves the accuracy of calibration. 2) Blue-labeled molecules (Fig. 1a) are separated with higher accuracy than red-labeled molecules (Fig. 1b), hence different dye labels influence the DNA flow differently. 3) The influence of dye label is higher than fluctuations between two experiments. 4) Choosing a single, suitable dye label combined with reference calibration and sample investigation in consecutive experiments (left-hand side of dark-blue curve in Fig. 1c) results in $S = 4 \times 10^{-4}$, enabling detection of single base-pair insertion/deletion in a lab-on-a-chip.



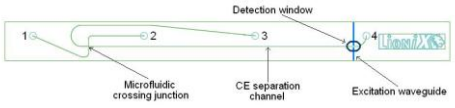
(a)



(b)



(c)



(d)

Fig. 1. 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). Linear and quadratic fits (solid lines) applied to data of experiment 1. (c) Standard deviation of measured data from quadratic fit function. (d) Schematic of optofluidic chip.

References

[1] C. Dongre *et al.*, Electrophoresis 31 (2010) 2584–2588.
[2] R. Martínez Vázquez *et al.*, Lab Chip 9 (2009) 91–96.
[3] C. Dongre *et al.*, Lab Chip 11 (2011) 679–683.