Sub-base-pair Resolution During DNA Separation in an Optofluidic Chip

Markus Pollnau, Manfred Hammer, Chaitanya Dongre, Hugo J. W. M. Hoekstra

Integrated Optical Microsystems Group, MESA+ Institute for Nanotechnology,
University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
m.pollnau@utwente.nl

Abstract: 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}, thereby enabling micro-chip analysis of genetic illnesses with single-base-pair deletion/insertion.

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

Experimental: A commercial microfluidic chip (LioniX BV), as displayed in Fig. 1, 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,[1,3] each set excited exclusively by either of two lasers power-modulated at different frequencies of 17 Hz and 31 Hz and launched through the optical waveguide, their fluorescence detected by a sensitive photomultiplier, and blue and red signals distinguished by Fourier analysis.[3] The results are displayed in Fig. 2.

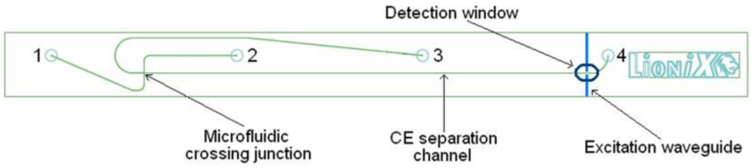

Fig. 1: Schematic of optofluidic chip.

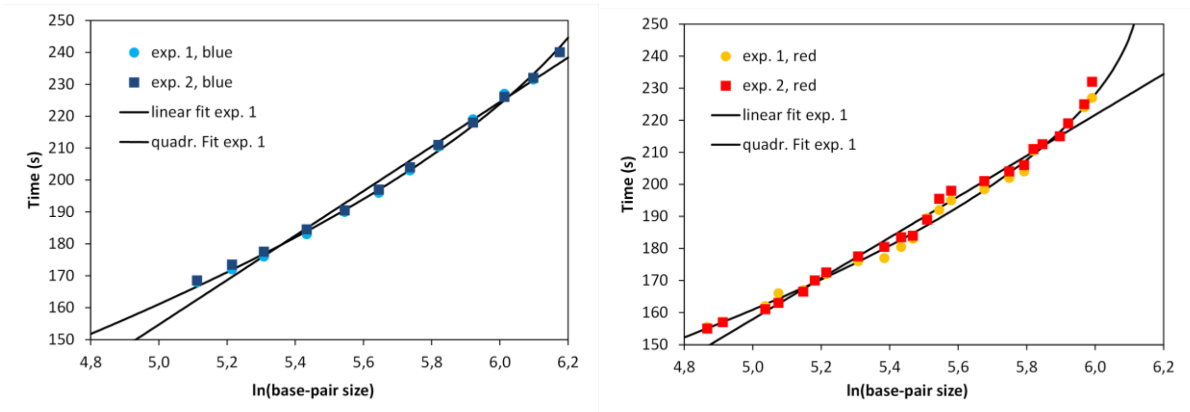

Fig. 2: Migration time (linear scale) vs. DNA base-pair size (logarithmic scale) of (a) 12 blue-labeled and (b) 23 red-labeled DNA molecules simultaneously migrated and separated in experiment 1 (circles) and experiment 2 (squares).

Data analysis: Different calibration strategies for the dependence of migration time on base-pair size in a given experimental situation 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. Results of the analysis are displayed in Fig. 3.

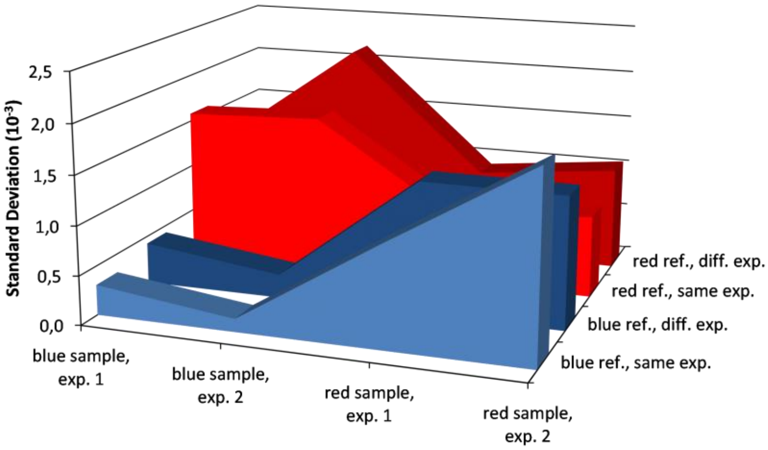

Fig. 3: Standard deviation of measured data from quadratic fit function.

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: Choice of a suitable dye label, combined with reference calibration and sample investigation in consecutive experiments, 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.

References

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