DNA separation and fluorescent detection in an optofluidic chip with sub-base-pair resolution

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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/deletions from DNA fragments in the diagnostically relevant range of 150−1000 base-pairs requires a sizing accuracy of $S < 10^{-3}$. Here we demonstrate $S = 4\times10^{-4}$. A microfluidic chip was post-processed by femtosecond-laser writing of an optical waveguide. 12 blue-labeled and 23 red-labeled DNA fragments were separated in size by capillary electrophoresis, each set excited by either of two lasers power-modulated at different frequencies, their fluorescence detected by a photomultiplier, and blue/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 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. From the results we conclude: Applying quadratic instead of linear fit functions improves the calibration accuracy. Blue-labeled molecules are separated with higher accuracy. The influence of dye label is higher than fluctuations between two experiments. Choosing a single, suitable dye label combined with reference calibration and sample investigation in consecutive experiments results in $S = 4\times10^{-4}$, enabling detection of single base-pair insertion/deletion in a lab-on-a-chip.

Keywords: DNA separation, optofluidic chip, capillary electrophoresis, fluorescent detection, single base-pair insertion/deletion, sub-base-pair sizing accuracy

1. INTRODUCTION

A lab on a chip [1-4] squeezes the functionalities of a biological/chemical laboratory onto a single substrate through a network of microfluidic channels, reservoirs, valves, pumps and sensors. Its advantages are speed of analysis, low sample consumption, and measurement automation and standardization. Capillary electrophoresis (CE) is a powerful method for biomolecule separation and analysis. The sorting and sizing of DNA molecules within the human genome project [5] has been enabled largely by CE separation and analysis [6]. The human genome project has also lead to the genetic mapping of various human illnesses [7]. On-chip integration of DNA sequencing [8-11] as well as genetic diagnostics [12,13] have become feasible. Laser-induced fluorescence is the most popular microchip CE monitoring technique, allowing a low limit of detection of 210 fM [14] using visible fluorescent dye labels.

Many attempts have been made to integrate micro-optical components in microfluidic labs on a chip to perform on-chip optical detection [15-27]. In addition to the integration of optical waveguides, the use of the microfluidic channel itself as a liquid-core optofluidic waveguide [25,26,28,29] has been explored. Integrated optical waveguides allow to confine and transport light in the chip, directing it to a small volume of the microfluidic channel and collecting the emitted/transmitted light, as has recently been applied to monitor on-chip DNA sequencing using zero-mode waveguide sensors [9,30] in a now commercialized DNA sequencer [11]. Among the different waveguide fabrication processes, the technique of femtosecond (fs-) laser writing of waveguides in glass [31-43] allows for simple post-processing of commercial microfluidic chips.

In this contribution, we demonstrate CE-based DNA analysis in an optofluidic chip with sub-base-pair resolution of low concentrations of permanently, exclusively end-labeled DNA molecules.

2. EXPERIMENTAL

A schematic of the commercial microfluidic chip (LioniX BV) [44] is displayed in Fig. 1. The microfluidic channel network and reservoirs were patterned photolithographically and wet-etched in fused silica glass and then sealed off by bonding another piece of fused silica glass on top. The chip has dimensions of 55 mm × 5.5 mm × 1 mm and the microfluidic channels have a cross-section of ~110 µm width and ~50 µm depth [44]. The optical waveguide was inscribed into the bulk of the fused silica chip by fs-laser writing [40] at a translation speed of 20 µm/s, using a Ti:Sapphire laser operating at 800 nm wavelength with 150 fs, 4 µJ pulses at a repetition rate of 1 kHz [41-43]. Employing tunable, astigmatic beam shaping [39], an elliptical cross section of the written waveguide was obtained, with a major diameter of ~50 µm in the vertical direction, while the minor diameter in the horizontal direction is ~12 µm in order to retain a high spatial resolution along the direction of DNA migration and separation.

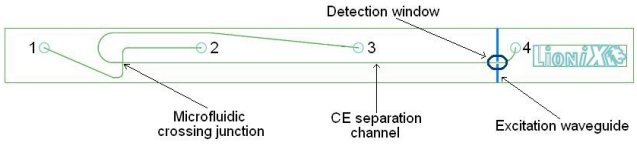

Figure 1. Schematic of the optofluidic chip [44] showing reservoirs 1–4, sample injection channel (reservoir 1 → reservoir 2) and CE separation channel (reservoir 3 → reservoir 4), as well as the integrated optical waveguide and detection window.

Application of a high voltage forced the analyte molecules to migrate into the CE injection channel from sample reservoir 1 to waste reservoir 2 (see Fig. 1). By switching the voltages at all four reservoirs simultaneously a well-confined plug containing the mixture of analyte molecules – with a volume of ~605 picoliters at the crossing junction of the two microfluidic channels – was injected into the CE separation channel, from the microfluidic crossing junction toward waste reservoir 4, and the analyte molecules contained in the plug volume were separated according to their size [45-48].

Two sets of DNA molecules with known base-pair sizes were permanently end-labeled with different dyes to identify their origin. The 12 blue-labeled and 23 red-labeled DNA fragments were separated in size by microchip CE, 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 [48,49]. The experiment was then repeated.

3. RESULTS AND DISCUSSION

The 12 blue-labeled DNA molecules exhibit a clearer temporal migration behavior, less deviation of individual DNA molecules from this behavior, and the behavior is better reproduced in the second experiment. We fitted the data of $\ln(S_{bp})$ of the base-pair size S_{bp} as a function of time with a quadratic dependence.

We tested different calibration strategies for the dependence of migration time on base-pair size in a given experimental situation: (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; (b) use the same molecular set as reference and sample 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; (d) also self-calibration in the same experiment was analyzed. Results of the analysis are displayed in Fig. 2.

From the analysis we conclude the following: (a) Applying quadratic instead of the usual linear fit functions improves the accuracy of calibration. (b) Blue-labeled molecules are separated with higher accuracy than red-labeled molecules, hence different dye labels influence the DNA flow differently. (c) Different dye labels affect the formation and microfluidic flow of individual DNA plugs more severely than variations between consecutive experiments. (d) Choosing a single, suitable dye label, combined with reference calibration and sample investigation in consecutive experiments results in a sizing accuracy of $S = 4 \times 10^{-4}$, thereby enabling detection of single base-pair insertion/deletion in a lab-on-a-chip.

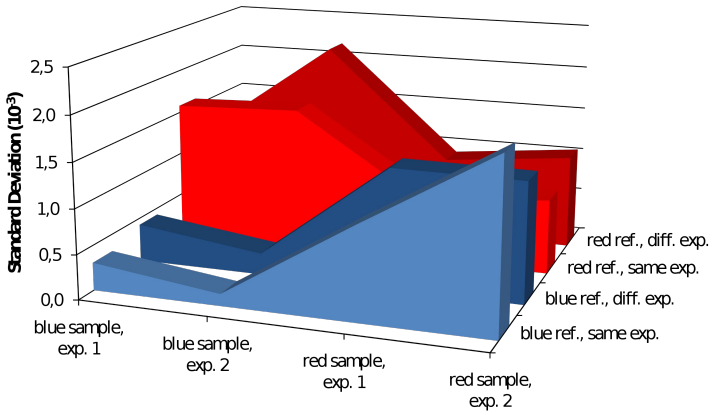

Figure 2. Standard deviation of measured sample data.

4. SUMMARY

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.

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