# MICROFLUIDIC DEVICES FOR FRACTIONATION OF DNA FRAGMENTS

**Kai Sun[1], Zheyu Li[1], Kosei Ueno[2], Nanqi Ren[1], Hiroaki Misawa[2]**

*[1] State key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, China,*
*[2] Research Institute for Electronic Science, Hokkaido University, Japan*

**ABSTRACT**

The fraction collection of DNA fragments for studying gene expression, suffered from technical difficulties, including those related to the accurate capture of fast moving targets and pure collection under conditions of insufficient separation.

We introduces a new concept of simultaneous space sampling for capturing targets in complicated situation, like overlapping fragments. Ten parallel extraction channels which covered 1.5 mm long sampling ranges were used to facilitate the capturing of fast moving fragments. Furthermore, the space-sampling extraction made it possible to acquire pure collection, even from partly overlapping fragments that had been insufficiently separated after a short electrophoretic run. Three fragments differed in size by one base bases, were simultaneously collected within 10 min.

**KEYWORDS**

space sampling , Fractionation, Microfluidics, Reconstruction.

## INTRODUCTION

Microchip is selected as an ideal tool for fraction collection, because of flexible 2D or 3D structure, real time observation, less contamination, fast analysis time and automation. The primary issues now such as, high efficiency, capturing of fast moving components, and cross contamination between neighboring components need to be solved.

Here we introduce a novel electrophoretic chip device for fractionation of desired DNA fragments. Collection methods utilizing a lab-on-a-chip can be categorized into one of the following four classes: (i) a main separation channel combined with a perpendicular extraction channel for single target collection [1]; (ii) more transfer channels connected to the single perpendicular extraction channel for multiple target collections [2]; (iii) isolated extraction channels connected to the main separation channel to perform multiple collections for reducing cross-contamination between collections [3]; and (iv) a novel matrix composed of small-diameter posts, with the distance between the posts providing continuous-flow fraction collection for large DNA fragments simultaneously at different locations, thus saving time and increasing recovery [4]. However, it was difficult for all these methods to isolate the DNA fragment from the others that differed in size by one base with few minutes. Our method based on a signal processing theorem that allows simultaneous space sampling succeeded this high-fidelity fractionation within 10 min.

We proposed a new concept of simultaneous space sampling for capturing targets in complicated situation, like overlapped fragments or fragments with fast mobility. The focused laser spot was located at the intersection of Channel 10 and the main separation channel. When the selected target fragments passed the detection point, the extraction was performed. All separation electrodes were isolated from the electric field, bias potential is applied to Channel 10 to extract the selected fragments, all ten extraction channels are applied with the same potential for simultaneous sampling of the targets. The recovery samples were PCR-amplified, then were size-checked. The reconstruction of the PCR amplified recovery can be acquired, which shows the distribution of each isolated band.

In contrast to the previous single extraction collection or sequential multiple collection techniques, our method provide the capacity for high-resolution collection at insufficient separation lengths with higher efficiency.

## EXPERIMENT

The microfluidic structure (Fig. 1) was fabricated on a 4-inch diameter quartz wafer using standard photolithography and wet-etching techniques [2]. The chip consisted of 24 3-mm-diameter reservoirs, a typical injector unit, a 70-mm-long straight separation channel, and ten parallel channels for the extraction and retrieval of the target. The channels were 60 μm wide and 25 μm deep. The interval between parallel channels was 100 μm. In this fractionation study, extraction sampling was performed simultaneously using ten parallel channels with identical spacing when the desired fragment arrived at the sampling zone. According to Nyquist–Shannon sampling theorem, we introduced the simultaneous space sampling method (Fig. 2). Sampling rate = 1/ *S*, where *S* is the spacing between two neighboring extraction channels. Sampling rate is based on the spacing, the spacing between our two channels is 160um, so the sampling rate is about 6.25/mm, it means that a 1-mm-wide Gaussian peak at baseline will be isolated into 6 slices. There are 10 isolated channels in our structure. So, a peak with a width of less 1.6 mm can be reconstructed by our sampling structure. It offers several advantages over previous methods, which include, a wide sampling range, or cover distance, in separation direction from the first channel to the final channel (i.e., a 500-μm-wide peak at the velocity of 150 μm/s needs more than 10 s to pass through this 1.5-mm-wide window; hence, a small shift in the time of capture will not lose the peak) and the capacity for pure collection (i.e., overlapping peaks can be sliced into many pieces, and the pure fraction of the target can be distinguished from these recoveries because of different distributions of components in the overlapping parts, which simplifies the collection



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of the peaks that are not separated sufficiently). Fragments of 180, 181 and 182 bases, were simultaneously collected, and then the recovered DNA was PCR-amplified and assessed by capillary electrophoresis (CE) analysis (Fig. 3). The 181-base target was shown to be isolated in a 70-mm-long separation length within 10 min, in contrast to the >50 min required for the 300-mm-long separation channel in our precious work [5].

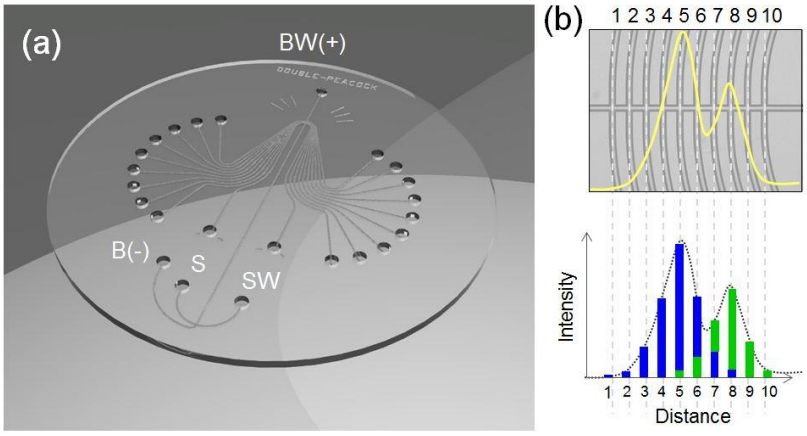


Figure 1. 3D schematic drawing of a 4-inch -diameter microfluidic device

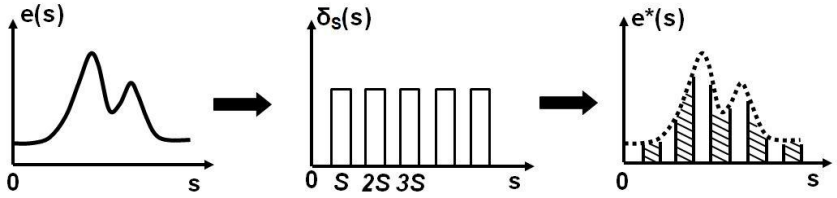


Figure 2. Illustration of simultaneous space- sampling

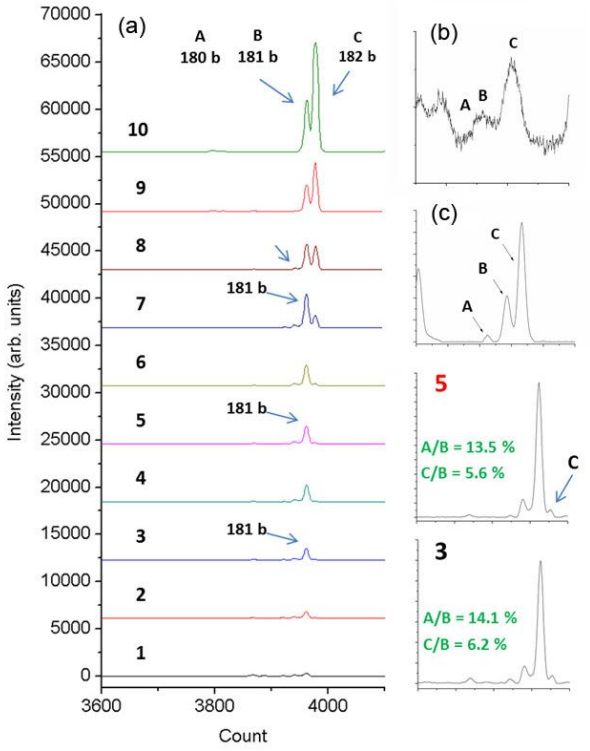


Figure 3. CE analysis of the PCR-amplified on-chip fractionated samples (a); separation of the samples before fractionation in the chip (b) and in the 36cm long capillary (c). Numbers 1 to 10 denote the channel number of the comb structure. The target fragment is 181 b.

This method provides the capacity for high-resolution collection at insufficient separation lengths. Furthermore, it provides a roadmap for the reconstruction of the separation to explore the separation mechanism (Fig. 4). This reconstruction shows the distribution of each isolated band. This is very meaningful to understand the overlapping in insufficient separation. The fractionation-integrated electrophoretic microfluidic device will also provide an important reference and could become a new and powerful analytical tool for bioanalysis and related applications.

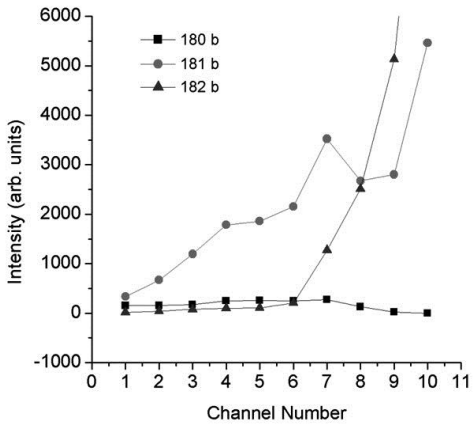


Figure 4. A reconstruction of the fragment distribution of 180, 181 and 182-b fragments after a 70-mm separation according to the data in Fig. 3.

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**CONTACT**

Kai Sun 86-451-86289216 or ksun@hit.edu.cn