Band-broadening suppressed effect in long turned geometry channel and high-sensitive analysis of DNA sample by using floating electrokinetic supercharging on a microchip

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A featured microchip owning three big reservoirs and long turned geometry channel was designed to improve the detection limit of DNA fragments by using floating electrokinetic supercharging (FEKS) method. The novel design matches the FEKS preconcentration needs of a large sample volume introduction with electrokinetic injection (EKI), as well as long duration of isotachopheresis (ITP) process to enrich low concentration sample. In the curved channel [~ 45.6 mm long between port 1 (P1) and the intersection point of two channels], EKI and ITP were performed while the side port 3 (P3) was electrically floated. The turn-induced band broadening with or without ITP process was investigated by a computer simulation (using CFD-ACE+ software) when the analytes traveling through the U-shaped geometry. It was found that the channel curvature determined the extent of band broadening, however, which could be effectively eliminated by the way of ITP. After the ITP-stacked zones passed the intersection point from P1, they were rapidly destacked for separation and detection from ITP to zone electrophoresis by using leading ions from P3. The FEKS carried on the novel chip successfully contributed to higher sensitivities of DNA fragments in comparison with our previous results realized on either a single channel or a cross microchip. The analysis of low concentration 50 bp DNA step ladders ($0.23 \mu\text{g/ml}$ after 1500-fold diluted) was achieved with normal UV detection at 260 nm. The obtained limit of detections (LODs) were on average 100 times better than using conventional pinched injection, down to several ng/ml for individual DNA fragment. © 2010 American Institute of Physics. [doi:10.1063/1.3366719]

I. INTRODUCTION

Electrophoretic separations on microfluidic devices, conventionally termed microchip electrophoresis (MCE), have obtained an exciting development since the pioneering work done by Manz and Harrison.¹⁻³ MCE owns the advantages as rapid analysis, reduction in chemicals, as well as to integrate multiple analytical processes together due to the technological development for chip making and diversity designs of reservoirs and channels.^{4,5} MCE has been successfully used to separate biopolymers, such as labeled amino acids,⁶ DNA restriction fragments,^{7,8} polymerase chain reaction (PCR) products,^{9,10} short oligonucleotides,¹¹ and even cells.^{12,13} In addition, the capability of MCE to analyze organic pollutants¹⁴ and pharmaceutical drugs¹⁵ has also been explored and proved. In practice, the injected and detected sample volumes for MCE are at

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picoliter level or even lower, which match the analytical requirement of low consumption of samples, running cost saving, and good resolution in a short migration distance of the microchip. Unfortunately, this may result in detection limits. Two concepts to overcome this problem have been demonstrated: the use of high-sensitive detectors and the coupling of preconcentration techniques on microchips. mass spectrometry (MS) is the most powerful detection method because of its specificity, high sensitivity, and structure identification. Integrating MS to microchip is capable of low concentration bioanalysis,¹⁶ but the major challenge is to develop a stable and efficient electrospray ionization interface to couple microchip with MS. So far, more reports on sensitivity improvement are concerned with laser-induced fluorescence (LIF) detection.^{17,18} However, expensive laser systems, accessories, as well as pre- or postcolumn labeling of nonfluorescent analytes are required for LIF detection. Besides using above advanced detectors, an alternative for improving the sensitivity is to transfer preconcentration techniques already existing in conventional capillary electrophoresis (CE) field to MCE format. On diversity microchips, stacking techniques based on chromatographic and electrophoretic principles were developed. The progress of on-chip preconcentration methods was reviewed by Sueyoshi *et al.*¹⁹

Electrokinetic injection (EKI) is a simple and convenient sample injection way, which is easy to be implemented on a microfluidic chip.²⁰ However, EKI always accompanies the band broadening that is mainly caused by migration length and field strength difference in a microchannel. Especially aiming to a large amount of sample loading or the analytes migrating through the curved channel, the band broadening would become more serious and deteriorate the final separation. A couple of calculations and theoretical models of the turned geometry channel were studied in order to reduce the spreading of a band because the turned design is always used to offer long microchannel in a compact microchip.^{13,21–23} Culbertson *et al.*²⁴ first studied the band broadening in constant radius turns, and illustrated the band spreading was determined by the angle and width of the turn. Molho *et al.*²⁵ designed a turned geometry not with the constant radius, made the turned path length inside, and outside was the same in the simulation. So the broadening in 90° and 180° turns was suppressed by eliminating the difference in electric field at the turned part. In practice, the ideal mathematical model for dispersion-reduction geometries would be difficult to be manufactured.

As well known, isotachopheresis (ITP) is a characteristic electrophoretic mode, which is capable to sharpen a long and diluted sample plug into a focused zone. With the suitable selection of the leading and terminating ions, transient ITP (tITP) could contribute to on-chip sample stacking through special design of microchannels.^{26,27} The coions in background electrolyte (BGE) as the role of leading and terminating ions (named medium-induced tITP) could reduce the steps for buffer/sample replacement. Hirokawa *et al.*^{28,29} termed an approach combining EKI with tITP for electrokinetic supercharging (EKS), which has higher sensitivity than sole EKI or tITP applied since tITP can remedy the drawbacks of EKI. By adopting on-chip EKS, the limit of detections (LODs) of DNA fragments and sodium dodecyl sulfate (SDS)-protein complexes were improved 10–30 times better than those obtained by pinched injection on a conventional cross chip.^{30–32} The same group recently developed a novel sample injection method named floating EKS (FEKS) on the cross chip because two branch channels and ports were electrically floated in the EKI and ITP steps.³³ The possible sample leakage to the branch channels and subsequent recovery into the separation channel were detailed by using computer simulation results. The FEKS method successfully contributed to high resolution and short analysis time on a cross microchip owing to rapid switch from ITP to zone electrophoresis (ZE) separation.

The goal of this work is to develop a specific microchip that could afford sample overloading by EKI and long duration of ITP, such two steps are essential for strengthening FEKS preconcentration. In addition, it first elucidated a significant effect on reducing band broadening by ITP. The novel chip owns three large volume ports and a long curved channel. In this new design, the long curved channel connecting ports 1 and 2 (abbreviated as P1 and P2, hereafter) was used for sample injection, preconcentration, and final separation. Port 3 (P3 at the end of branch channel) would offer leading ion for the migration switching from ITP mode to ZE separation. Furthermore, we also simulated the behavior of migrating zone with or without ITP stacking in the curved channel,

FIG. 1. (a) Picture and (b) schematic of the designed microchip. Functional distribution of three reservoirs for FEKS method: port 1 is the sample reservoir; ports 2 and 3 are the reservoirs for BGE. See text for detailed dimension.

where the serious band broadening always occurs because the potential gradient is different in the radial direction. It was demonstrated that ITP technique significantly benefited to suppress turn-induced broadening and keep stable boundary in the U-shaped geometry channel. So ITP could be a useful approach to conduct well-defined sample plug in microfluidic chip. The obtained LOD for the analysis of DNA fragments with normal UV detection was at ng/ml, which is almost comparable to tITP preconcentration combined with LIF detection.³⁴

II. MATERIALS AND METHODS

A. Microchip and apparatus

We designed a microchip with two features to enhance FEKS preconcentration. The three reservoirs (P1, P2, and P3) are of big capacity (5 μ l) to offer sample overloading by EKI. Five U-shaped turns with a straight channel segment achieved 45.6 mm long channel between P1 and the intersection point. Here, the channel was able to provide long ITP time to ensure enough electric charge for preconcentration. The used silica-glass microchip made by Microchemical Technology Co. Ltd. (Kanagawa, Japan) is presented in Fig. 1. The capacity and length are 1.7- and 6-folds greater than those of the cross microchip from Shimadzu Co. (Kyoto, Japan),³³ but the chip size (12.5 $\times$ 35 mm²) was kept the same; thanks to the turn geometries. So, the manufactured chip could be directly used on the MCE-2010 system from Shimadzu. The system consisted of a

FIG. 2. Frame of a turn geometry microchip for 2D computer simulation. [(a) and (d) segments] Quarter-circle pipe for 90° turn. [(b) and (c) segments] Two quarter-circle pipes connected with a short capillary ($100 \times 100 \mu\text{m}^2$) for 180° turn.

linear imaging UV detector [a 1024 channel photodiode array with an effective length of 25 mm and a D2 lamp, marked as yellow part in Fig. 1(b)], which was located along the separation channel from the intersection. The width and depth of the microchannel were 110 and 50 μm , respectively. P1 was used as the analyte reservoir. P2 and P3 were used as BGE reservoirs. The lengths from intersection to P2 and P3 are 33.0 and 7.5 mm, respectively. After the changing in original power supply, three Pt electrodes were inserted into the suited reservoirs to achieve FEKS stacking and zone separation.

B. DNA sample and chemicals

The DNA sample was a mixture of 50 bp step ladders (Promega Co., Madison, WI, USA). The standard marker consisted of 16 DNA fragments ranging from 50 to 800 bp in exactly 50 bp increments. The original concentration of the DNA sample was 340 $\mu\text{g}/\text{ml}$ including starting material (1800 bp DNA), in which the concentration of 800 bp component was two to three times higher than that of other components. Chemicals used for the buffer preparation and microchip coating were purchased from different companies. The sieving matrix hydroxyethyl cellulose (HEC) (average molecular mass of 250 000 Da) was from Aldrich Chemical Co. (Milwaukee, WI, USA). Tris(hydroxymethyl)aminomethane (tris), glycine, hydrochloric acid, boric acid, ethylenediaminetetraacetic acid (EDTA), acrylamide, and ammonium persulfate were from Sigma-Aldrich (Tokyo, Japan). Tetramethylethylenediamine (TEMED) was from Tokyo Kasei (Tokyo Japan), and 3-methacryloxypropyl-trimethoxysilane (MAPS) was from Chisso (Tokyo, Japan). The ultrapure water was obtained from a Millipore purification system (Tokyo, Japan). The buffer systems were developed specifically for FEKS preconcentration and separation of DNA fragments. The BGE was composed of 2% (w/v) HEC and 50 mM HCl (at pH 8.1 adjusted by tris), which also worked as the leading electrolyte (LE). The chloride ion played the role of the leading ion for ITP preconcentration. The terminating electrolyte (TE) contained 20 mM glycine as the terminator at pH 8.1 adjusted by adding tris.

C. Computer simulation model

A multiphysics software, CFD-ACE+ (version 2006, CFDRC, Huntsville, AL, USA), was used for computer simulation to explore the migrating behavior of the analytes with or without ITP stacking in the curved channel. Figure 2 shows the frame of a two-dimensional (2D) model and

dimension based on a finite element method. To reduce the calculation time, the assumed channel length and volume of reservoirs were smaller than those of the real microchip, however, the channel width ($100\text{ }\mu\text{m}$) was almost the same as the real value ($110\text{ }\mu\text{m}$). As shown in Fig. 2, three ports were connected with two channels, and the electrodes were set at the end wall of each port. The size of meshes in each port was $50\times 50\text{ }\mu\text{m}^2$, and that was $10\times 20\text{ }\mu\text{m}^2$ in channels. Regarding the turn geometry segments with the constant radius, (a) and (d) for 90° turn were quarter-circle pipes, (b) and (c) for 180° turn were two quarter-circle pipes connected with a short capillary ($100\times 100\text{ }\mu\text{m}^2$). Two different curvatures of turn geometry, the radius (r), at 20 and $100\text{ }\mu\text{m}$, respectively, were simulated to compare the migration performance of ITP-stacked zone. The mobilities of five model anions were at $50\times 10^{-5}(\text{m}50^-)$, $40\times 10^{-5}(\text{m}40^-)$, $30\times 10^{-5}(\text{m}30^-)$, $20\times 10^{-5}(\text{m}20^-)$, and $10\times 10^{-5}(\text{m}10^-)\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$. Initial concentration was assumed at 0.2 mM for each analyte (buffered by tris to pH 8.1). The used BGE (LE) was a mixture of 50 mM HCl [$\text{m}_0(\text{Cl}^-)=79.1\times 10^{-5}\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$] and 100 mM tris ($\text{m}_0=29.5\times 10^{-5}$, $\text{pK}_a=8.076$). The TE was comprised of a model anion with mobility $5\times 10^{-5}\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$ and the initial concentration was 50 mM (buffered by tris to pH 8.1). The electro-osmotic flow (EOF) was not assumed here. Time spacing (dt) was 0.25 ms (4000 calculation steps for 1 s simulation). It took $\sim 600\text{ h}$ to simulate a 3.5 s electromigration process.

III. RESULTS AND DISCUSSIONS

A. Microchip coating

The inner surface of microchip was appropriately treated with linear polyacrylamide (LPA) coating method for the purpose of improving the separation performance and reproducibility of DNA analysis.³⁵ A syringe pump was set at the rate of $20\text{ }\mu\text{l}/\text{min}$ to introduce the solutions into the microchannels in the coating process. Three major steps were carried on to achieve LPA coating: (1) The microchannels were first rinsed with 1M NaOH for 20 min and 0.1M NaOH for 60 min. The channels were sealed and kept at ambient temperature (around 25°C) for 3 h, and then rinsed by ultrapure water for 20 min. (2) A solution containing 1% (v/v) MAPS and 2% (v/v) methanol was flushed through the channels for 60 min, and the channels were sealed for one night. The channels were then rinsed with methanol for 20 min and dried at 110°C on a hotplate for 30 min. Thus, the bifunctional silane reagent was attached to the inner surface. (3) The 3% (w/v) acrylamide solution, 0.1% (v/v) TEMED, and 0.1% ammonium persulfate were prepared in $1\times\text{TBE}$ buffer (89 mM tris, 89 mM boric acid, and 2 mM EDTA). After the degassing, the solution was immediately introduced into the channels by syringe pump. The channels were then sealed for 30 min at the ambient temperature for the polymerization reaction. After reaction, the solution was pressurized out and the channels were flushed with ultrapure water for 20 min. After these procedures, the microchannels were LPA coated and ready for DNA separation. It was proved that such coating process was effective to obtain good reproducibility for a long lifetime.

B. FEKS procedure on three-port microchip

We first developed FEKS on a conventional cross geometry microchip.³³ Here, the analytical procedure could be also adopted on such novel three-port chip. After all the channels and ports were filled with the BGE (LE), the reservoir P1 was replaced by the DNA sample solution, which was introduced toward P2 through EKI. Then P1 was replaced by TE, which was also electrokinetically introduced for starting ITP preconcentration. The reservoir P3 was left floating during EKI with ITP procedure. Owing to the real-time imaging UV detector, we can directly observe the movement of sample zone in detection window. Once the stacked zone migrated beyond the intersection point, the driving voltage applied on P1 and P2 should be switched off. Subsequently, the voltage was applied on P3 and P2 while keeping P1 electrically floating; so the leading ions in P3 migrated to P2 simultaneously leading the ITP-focused zone into ZE separation manner. For FEKS, the longer duration of ITP (the greater electric charge), the better stacking effect depending on the molar amount and mobility difference in the analytes. So the long preconcentration channel layout (curved part) is necessary for obtaining good preconcentration and improving detection

FIG. 3. Simulated profile for the band broadening of $m50^-$ in the turn. [(a) and (b)] ITP-stacked zone; (c) without ITP process. The inside turn radius r is at (a) $20\ \mu\text{m}$ and [(b) and (c)] $100\ \mu\text{m}$. The channel width is $100\ \mu\text{m}$.

limit. Because most of the TE zone could be skipped and left between P1 and the intersection, the leading ions from P3 enables to transfer electrophoretic mode from ITP to ZE rapidly. It means that the sufficient resolution could be obtained. There would be some minor sample loss to the branch channel since the electrical dispersion, but the loss was almost recovered to the separation channel eventually after the voltage switch for ZE, which was previously validated in computer simulation.³³

C. ITP effect to reduce turn-induced broadening

The separation efficiency and detection sensitivity are significantly influenced by the shape and size of the sample plug that was introduced into the separation channel in MCE. A well-defined sample plug is not only determined by the voltage control but also by the geometries of microchannel. As a helpful tool, the computer simulation was used to evaluate the sample behaviors when traveling the 180° turn geometry of segment (b), as marked in Fig. 2. The turn-induced broadening of the analyte (e.g., $m50^-$) is presented in Fig. 3. Obviously, the inside band led the outside band at the turn; such deformation is caused by the racetrack effect. The electric field is different in the radial direction at turn geometry. The shorter path, the stronger electric field at the turn would cause the analyte rapidly migrating. Here, we compared the spreading status of ITP-stacked zone at two different radii at $20\ \mu\text{m}$ and $100\ \mu\text{m}$ in Figs. 3(a) and 3(b), respectively. The simulated results clarified the broadening was significantly dependent on the radius. The small radius [Fig. 3(a)] caused more serious band broadening in comparison with the big setting [Fig.

FIG. 4. The changing in zone length related to ITP effect: (a) with ITP; (b) without ITP in normal electrophoretic migration. The zone length was measured pre- and post-traveling through 180° turn during 0.7 s. The turn radius of channel was all at $100\ \mu\text{m}$.

3(b)] when the channel width was the same. In addition, the turn-induced deformation in Figs. 3(a) and 3(b) was self-corrected in the straight channel owing to ITP principle.³⁶ As known in ITP, the zone is stable as long as it migrates between correctly selected LE and TE, where its front and rear boundaries are stationarily sharp. The ITP-stacked zone is spreading in the turn due to nonuniform field strength toward radial direction. When the field strength is recovered and uniform in the straight channel, the zone could be back to the stationary state of ITP migration. Without ITP process, the zone was significantly deteriorated after the turn, as displayed in Fig. 3(c) even though r was $100\ \mu\text{m}$.

Two identical sample zones were set at the same position just before U-shaped turn (at $r = 100\ \mu\text{m}$), and then started to travel through the turn with or without ITP at the duration of 0.7 s. The zone length was measured in this process and presented in Fig. 4, which quantitatively demonstrated the effect of ITP on suppressing band broadening. The length of ITP-stacked band ($200\ \mu\text{m}$) was almost the same pre- and post-traveling through 180° turn owing to ITP principle in Fig. 4(a). Without ITP effect, the zone length was spreading to $545\ \mu\text{m}$ within the same period [Fig. 4(b)]. Apparently, Fig. 4 illustrated that ITP was a good broadening-reduction method for the turn geometries in microfluidic devices. Based on simulation results and chip size limitation, the turned radius was selected at $500\ \mu\text{m}$ for the making of the real chip, as displayed in Fig. 1. Two dyes (methylene blue and safranin O) were tested and directly observed in the manufactured microchip with charge coupled device (CCD) camera in the ITP status. The band length and the analyte boundary were kept well when the sample travels through the 180° turn geometry, as displayed in Fig. 5. In normal zone migration without ITP preconcentration, two dyes were electrically diffused in thin color and long length, so the photograph was not displayed since it is very difficult to distinguish sample from background.

D. LOD improved for DNA analysis by FEKS

To demonstrate FEKS preconcentration effect on the specific chip design, the used standard DNA sample was highly diluted in the ultrapure water. In FEKS procedure, the migration voltage was first added on P1 and P2 ($P1=0\ \text{V}$ and $P2=1200\ \text{V}$, $E=152.7\ \text{V/cm}$) while P3 was left floating. When the ITP-stacked zone just passed the intersection (into detection window), the

FIG. 5. Observation of methylene blue and safranin O in U-shaped turn in ITP mode by CCD camera. Sample concentration: 50 μ M. LE and TE: 50 mM NH_4OH and 10 mM HCl, respectively, at pH 4.8 adjusted by 2-ethylbutyric acid. Applied voltages: P1=800 V and P2=0 V while P3 was floating.

applied voltage was switched to P3 and P2 (P1 is floating, P3=0 V, and P2=430 V, $E=106.2$ V/cm). Thus, the leading ions migrating from the P3 would transfer ITP into ZE. Figure 6 shows the ITP-stacked zones of DNA samples in the separation channel at different concentrations (1:100, 1:600, 1:1200, and 1:2500 diluted samples). Thanks to the long curved channel, efficient ITP preconcentration resulted in a sharp sample zone despite long EKI time (150s). The half width of ITP-stacked band was around 400 μ m relative standard deviation (RSD=6% for three runs) for 1:1200 diluted sample, as in Fig. 6(c).

Microchip sometimes means the limited total channel length so the ITP stacking should be carefully controlled since the separation time and space needed for subsequent destacking. In previous studies, we had to shorten ITP time and sacrifice the preconcentration effect for earning sufficient resolution.^{30–33} In the present work, the turned geometry allowed long ITP process for the overloaded sample, which resulted in a low concentration limit. When adopting FEKS on a

FIG. 6. ITP-stacked zone for differently diluted 50 bp DNA stepladders. (a) 1:100, (b) 1:600, (c) 1:1200, and (d) 1:2500 (original concentration, 340 μ g/ml). BGE (LE), 2% (w/v) HEC, 50 mM HCl (at pH 8.1 adjusted by adding tris). TE, 20 mM glycine (at pH 8.1 adjusted by tris). Sample and TE injection were 150 and 320 s at P1=0 V and P2=1200 V, respectively, while P3 was floating. UV detection at 260 nm.

FIG. 7. Electropherograms of 1:100 diluted DNA sample ($3.4 \mu\text{g/ml}$) obtained by using FEKS on (a) three-port chip with long curved channel and (b) Shimadzu cross microchip. Optimized condition for (a): EKI time was 50 s and TE injection was 300 s ($P_1=0 \text{ V}$, $P_2=1200 \text{ V}$, and P_3 was floating). ZE separation time was 70 s ($P_3=0 \text{ V}$, $P_2=430 \text{ V}$, and P_1 was floating). Optimized voltages for (b) was described in Ref. 33, and times for EKI, TE, and ZE separation were 50, 30, and 70 s, respectively. Buffers as described in Fig. 6.

Shimadzu cross microchip, the LODs at $S/N=3$ were only 8–15 times enhanced, in comparison with the conventional pinched injection method. Figures 7(a) and 7(b) display the electropherograms of 1:100 diluted DNA sample ($3.4 \mu\text{g/ml}$) analyzed at the optimized FEKS conditions implemented in the current chip and a Shimadzu cross chip, respectively. Although the EKI time was the same (50 s), the injected amounts were apparently different between two types of chips due to the capacity of reservoirs and added electric charge. The ITP time was determined by the time of focused sample that just entered the detection window at 300 s [Fig. 7(a)] and 30 s [Fig. 7(b)], respectively. The conclusion is the greater sample introduction and better preconcentration can be achieved in the new microchip than that in either single channel microchip or cross microchip, as well as without sacrificing separation efficiency. Figure 8 is the trial analysis of highly diluted DNA sample (1:600 and 1:1500 diluted samples) by using FEKS preconcentration in the present novel chip. After 1500-fold dilution, the concentrations of DNA fragments of 100, 300, and 500 bp were 14.5, 6.6, and 2.7 ng/ml, respectively (the evaluation method has been previously described).³³ In Fig. 8, several ng/ml level DNA fragments could be detected on the

FIG. 8. Electropherograms of highly diluted DNA samples (a) 1:600 and (b) 1:1500, preconcentrated by FEKS on a three-port chip. The times of sample injection, TE injection, and ZE separation were 150, 320, and 70 s, respectively. The voltages and buffers as described in Fig. 6.

microchip with normal UV-visible detection at the conditions of long EKI (150 s) and ITP (320 s). The LODs (at $S/N=3$) were estimated at several ng/ml level that comparable to LIF detection,³⁴ and averagely 100 times better than the conventional pinched injection on cross microchip as detailed in Table I.

IV. CONCLUSION

The present study first used ITP to suppress turn-induced broadening, and illustrated DNA analysis on a novel design that owns big reservoirs and a long curved channel in a small compact chip that featured for FEKS preconcentration. Such design benefits to enhance the analytical sensitivity of DNA fragments with sample overloading by EKI and on-chip preconcentration by ITP process. For FEKS on the novel chip, the electrophoretic mode was rapidly changed from ITP to ZE owing to the elution of leading ions from its branch channel. The curvature of turn geometry was a significant factor that influenced the band broadening. As proved by the computer simulation, ITP was an effective approach to reduce the turn-induced broadening as well as to conduct well-defined zone in microfluidic device for subsequent separation. In real experiments, the highly diluted DNA sample (sub- $\mu\text{g/ml}$) could be focused into a sharp zone owing to ITP effect in spite

TABLE I. LODs obtained by FEKS on the novel microchip that compared pinched injecting and FEKS on the cross microchip.

DNA fragment (bp)	LOD on the cross chip ^a (ng/ml)		On the novel turn geometry chip		
	Pinched injection	FEKS	Concentration (ng/ml)	S/N	LOD (ng/ml)
100	2020	156	14.5	4.5	9.7
300	580	38	6.6	4.0	5.0
500	250	25	2.7	1.5	5.5

^aData from our previous publication (see Ref. 33).

of long time of EKI and traveling through five U-shaped turns. The average LODs for the DNA analysis by FEKS were improved around 100 times in comparison with the conventional pinched injection method on a cross microchip. The LODs were down to several ng/ml levels with UV detection at 260 nm that is close to those reported by tITP-LIF method. To the best of our knowledge, the LOD level may be the best value that was ever obtained by UV-visible detection for the DNA analysis in microfluidic devices.

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