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Selective extraction of size-fractioned DNA samples in microfabricated electrophoresis devices

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Abstract

We have designed and constructed a microfabricated device for separation of double-stranded DNA fragments using a crosslinked sieving medium and spatially selective extraction of the desired fraction. Based on measuring the width and spacing of migrating bands, a narrow side channel is constructed perpendicular to the separation channel to collect the DNA fragments of interest. This selective collection technique was tested using a 100 base pair double-stranded DNA ladder. We successfully demonstrate selective extraction of the desired fragment with minimal interference from the adjacent bands in an electric field of 31 V/cm. We also achieve extraction of multiple DNA fragments using an array of microelectrodes in this side channel. The device uses cross-linked polyacrylamide gel matrix, allowing the separation to be performed in a distance of 1 cm or less and at a low electric field strength. Together with on-chip electrode, this design is amenable to integration with reaction chambers into a single device for portable genetic-based analysis.
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Keywords: Instrumentation; Chip technology; DNA

1. Introduction

Electric field-mediated separations such as electrophoresis are generally performed at the analytical level. While these techniques are powerful, collection of electrophoretically separated fractions for future process can enhance and increase these techniques' performance. For instance, DNA analysis can use post-separation reactions such as southern blotting, polymerase chain reaction (PCR), restriction digestion reaction (RDR), and DNA sequencing. In one example, collection of size-separated oligonucleotides and the subsequent use of these fractions in a dot-blot assay have been previously demonstrated [1]. DNA fragments of interest can also be collected after separation for amplification by PCR and identification by DNA sequencing [2,3]. The isolation and collection techniques are critical steps toward successfully performing subsequent genetic assays on the extracted DNA fragments.

Isolation and collection of specific DNA fragments for subsequent assays is typically performed in a biology laboratory using slab gel electrophoresis followed by mechanical excision and extraction of

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the stained bands from the gel. Conventional agarose gels are capable of resolving DNA from approximately 150–80 000 base pairs (bp) at concentrations from 0.2 to 2.5%, and are the most commonly used medium for extracting specific DNA fragments. Crosslinked polyacrylamide gels ranging from 3 to 20% provide high-resolution separation in a range from 10 to 2000 base pairs [4]. These slab gel systems offer the ability to selectively extract DNA fragments appearing at any location in the fully separated sample. This spatial approach is of major importance especially when the pattern of sample bands is complex or not known. For example, a BAC clone DNA [100–150 kilo base pairs (kbp) length] can generate 10–100 fragments by restriction digestion, depending on the enzyme used. The entire electrophoretic pattern on the slab gel can be used to identify the band (or bands) to be extracted and processed further. Selectively extraction of DNA fragments from the middle of a complex band pattern has been widely used and provides considerable experimental flexibility.

In slab gels, the fragment is cut from gel after the electrophoresis run; consequently, this procedure is time-consuming and labor-intensive. By integrating the separation and collection of specified DNA fragments, capillary electrophoresis promises to reduce the personnel and time required to perform this procedure. Capillary electrophoresis typically employs replaceable mediums, such as, linear polyacrylamide, as the sieving matrix. Linear polyacrylamide gives consistent separation performance and is one of the most reliable sieving matrices used in CE. CE allows for extraction of fragments typically at the outlet end of the capillary and offers the potential for automation and integration [5]. Most collection methods in CE have employed sheath liquid arrangements at the capillary exit, where a stream of collection fluid flows around and transports the fractions into suitable collection vials. This continuous operation enhances the collection by minimizing band spreading due to interruptions [6,7].

Great strides have been made toward scaling-down existing capillary electrophoresis technology to the microchip level using photolithographic microfabrication [8–10]. Microfabrication technology uses planar glass, polymer or silicon substrates to produce microchip-based analytical tools. The resulting devices have the advantage of small reagent volumes, high-speed operation and parallel analysis with separation performance comparable to conventional gel electrophoresis [11,12]. By incorporating microfluidic components, this miniaturized format is amenable to system integration and multiplexing, leading to reduction in overall processing and analysis time [13–15]. Additionally, the spatially selective extraction technique used in slab gel electrophoresis could be integrated into this format.

The feasibility of collecting DNA fragments in chip-based systems was first demonstrated on a glass substrate [16]. Withdrawal of the selected sample is achieved by simple switching of the applied potentials to a channel network etched in the glass. The desired sample is driven towards the collection zone by the external electrodes and collected at one exit of the channel. Such a collection system was also fabricated on PDMS with external voltage control to extract one specific DNA fragment [17]. However, channel geometry and electric field at the intersection have not been addressed to optimize the performance. Fabricating multiple fraction collection zone at the end of the separation channel allows different fragments to be extracted for downstream processing [18]. The chip-based systems can also be readily incorporated with CE for high-precise fraction collection by electrokinetic manipulations and therefore enhance the collection efficiency with automatic operation [19]. Successful amplification of the collected sample by polymerase chain reaction shows the feasibility of performing subsequent analyses [20].

All of these chip-based systems employ the approach used in CE, collecting the sample at the end of the separation column. However, spatially selective extraction can provide more flexibility for analysis and would fully exploit the benefits of a miniaturized format. By this means, we can perform the same operation as in regular slab gel systems. Visualization of the entire electrophoresis pattern especially for unknown samples would be possible before extraction occurs. This type of operation would be advantageous especially when processing unknown samples.

In this paper, we describe such a chip-based electro-elution module for the separation of double-stranded DNA fragments using a crosslinked sieving

medium, and the spatially selective extraction of the desired fraction using on-chip electrodes. Instead of collecting the fragment at the exit of the separation channel, we design a side channel to selectively extract the target band after separation. Owing to the use of crosslinked polyacrylamide gels, this module can operate at much lower electric field strengths and offer high resolution separation in relatively shorter distances [21,22]. Unlike other chip-based systems, the desired sample is available as a spatially concentrated form and can be transported and stored at different location on a chip by employing microelectrodes. Such a system is fully amenable to integration with other individual components such as reaction chambers to create a portable genetic-based diagnostic device.

2. Experimental

2.1. Device fabrication and assembly

2.1.1. Silicon substrate

Fabrication of the microelectrodes was initiated with deposition of a thermal oxide layer (2000 Å) on the surface of a silicon wafer (500 μm thick, 10 cm diameter) to provide electrical insulation. As shown in Fig. 1a, the wafer was then spin-coated with a positive photoresist (Microposit SC 1827; Shipley, Marlborough, MA, USA) and patterned with the microelectrodes array. After the pattern was developed (MF-319 developer; Shipley), a 300-Å thick titanium metal layer followed by a 1000-Å platinum layer was deposited on this wafer by electron beam evaporation. The photoresist and the overlying metal layers were lifted off using acetone (complementary metal–oxide–silicon (CMOS) grade; J.T. Baker, Phillipsburg, NJ, USA) leaving only the microelectrode arrays. The wafers were then rinsed and dried.

2.1.2. Glass substrate

Fabrication of the glass channels (Fig. 1b) involved a similar process. First, metal layers of 600 Å chromium followed by 4000 Å gold were deposited on the surface of a borofloat glass wafer (500 μm thick, 100 mm diameter), which had been thermally annealed to provide smooth etched channel sidewalls. A positive photoresist (Microposit SC 1827; Shipley, Marlborough, MA, USA) was spin-coated and patterned using a channel mask and then developed. The metal layers were etched in a commercial gold etchant (Gold Etchant TFA, Transene) and chromium etchant (CR-14, Cyantek Corp., Fremont, CA, USA), respectively, leaving glass exposed in the locations where the channel network was to be etched. The accessible glass was then etched in a freshly prepared solution of hydrofluoric acid (49% HF, CMOS grade; J.T. Baker). The rate of etching was 7.0 μm/min and the etch-depth was measured using a stylus surface profilometer. After etching to the desired depth, the metal layers were removed using the corresponding etchants, and the wafer was rinsed in deionized (DI) water, air dried and oven-dried at 100 °C for 20 min. The final channel dimensions were 600 μm wide and 50 μm deep for the main channel and 200 μm wide for the side channel.

2.1.3. Device assembly

After individual devices on the substrate wafers were diced, holes (300 μm diameter) were drilled in the glass substrate to access the microchannels using an electrochemical discharge spark apparatus [23]. The glass channel was then bonded to the silicon substrate using a UV-cured optical adhesive (SK-9 Lens Bond, Summers Labs., Fort Washington, PA, USA). The bond was cured under a UV light source (365 nm) for 24 h. Electrical connections were made by wire bonding the assembled devices to printed circuit boards. Finally, the assembled devices were fitted with silicone electrophoresis buffer wells using 5-min epoxy (Loctite Corp., Rocky Hill, CT, USA). (Fig. 2a depicts assembly of the major component of these devices).

2.2. Sample preparation

Separation and extraction were performed using double stranded DNA ladders (Bio-Rad, Hercules, CA, USA) containing fragments separated by 100 bases ranging from 100 to 1000 bases in length. The double-stranded DNA ladders were labeled with YOYO-1 intercalating dye (Molecular Probes, Eugene, OR, USA) at a ratio of 5:1 bp/dye. A 1.0×

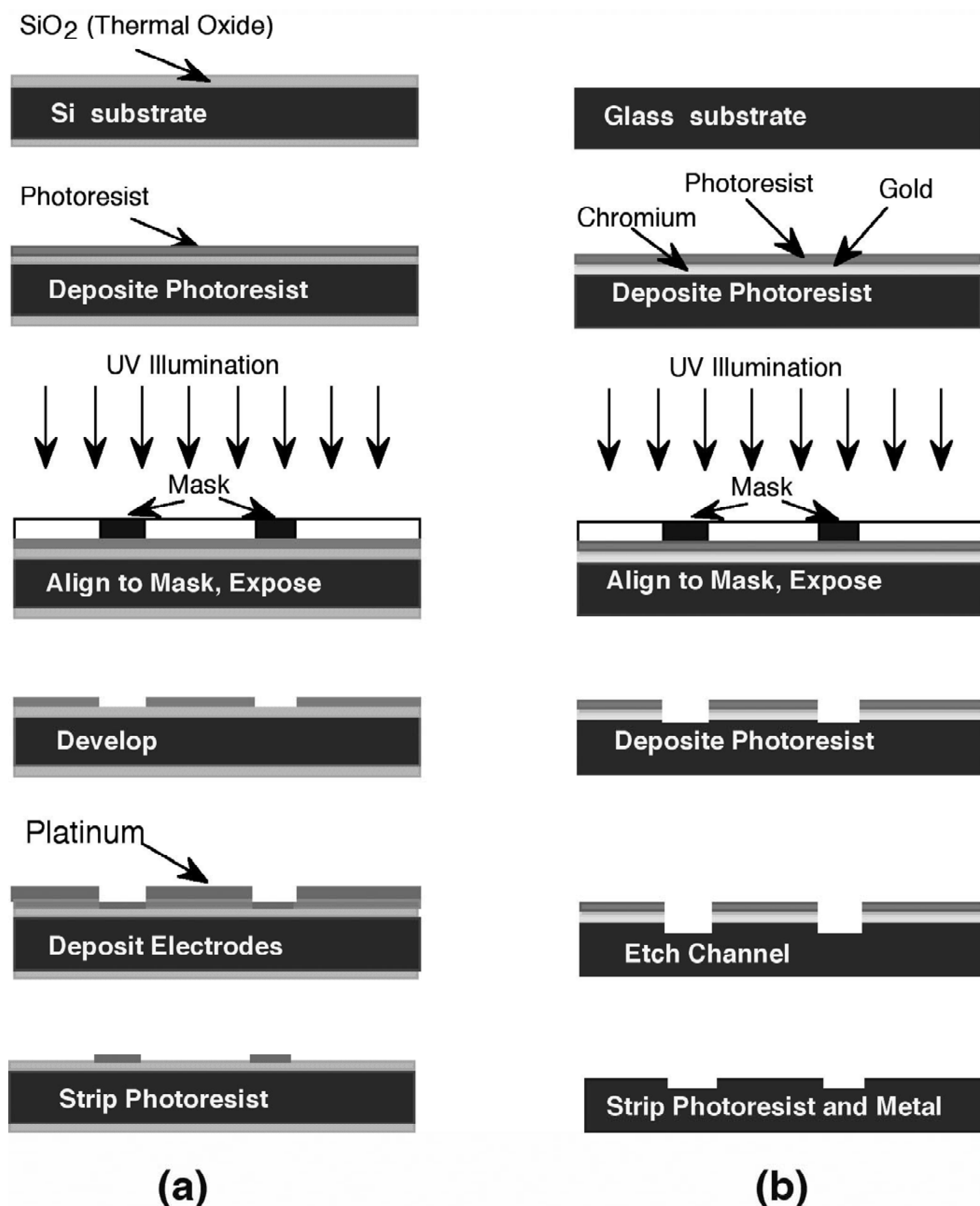


Fig. 1. Process flow for fabrication of (a) microelectrode and (b) microchannel network.

Tris–borate–EDTA (TBE) solution (Bio-Rad) was used as the running buffer.

2.3. Electrophoresis procedure

Prior to electrophoresis runs, the channels were rinsed with acetone, isopropyl alcohol and distilled/deionized water to remove particulate and organic matter. Following this cleaning procedure, the channels were rinsed with RainX AntiFog solution (Blue Coral, Cleveland, OH, USA) to promote uniform wetting on all channel surfaces by the gel reagents.

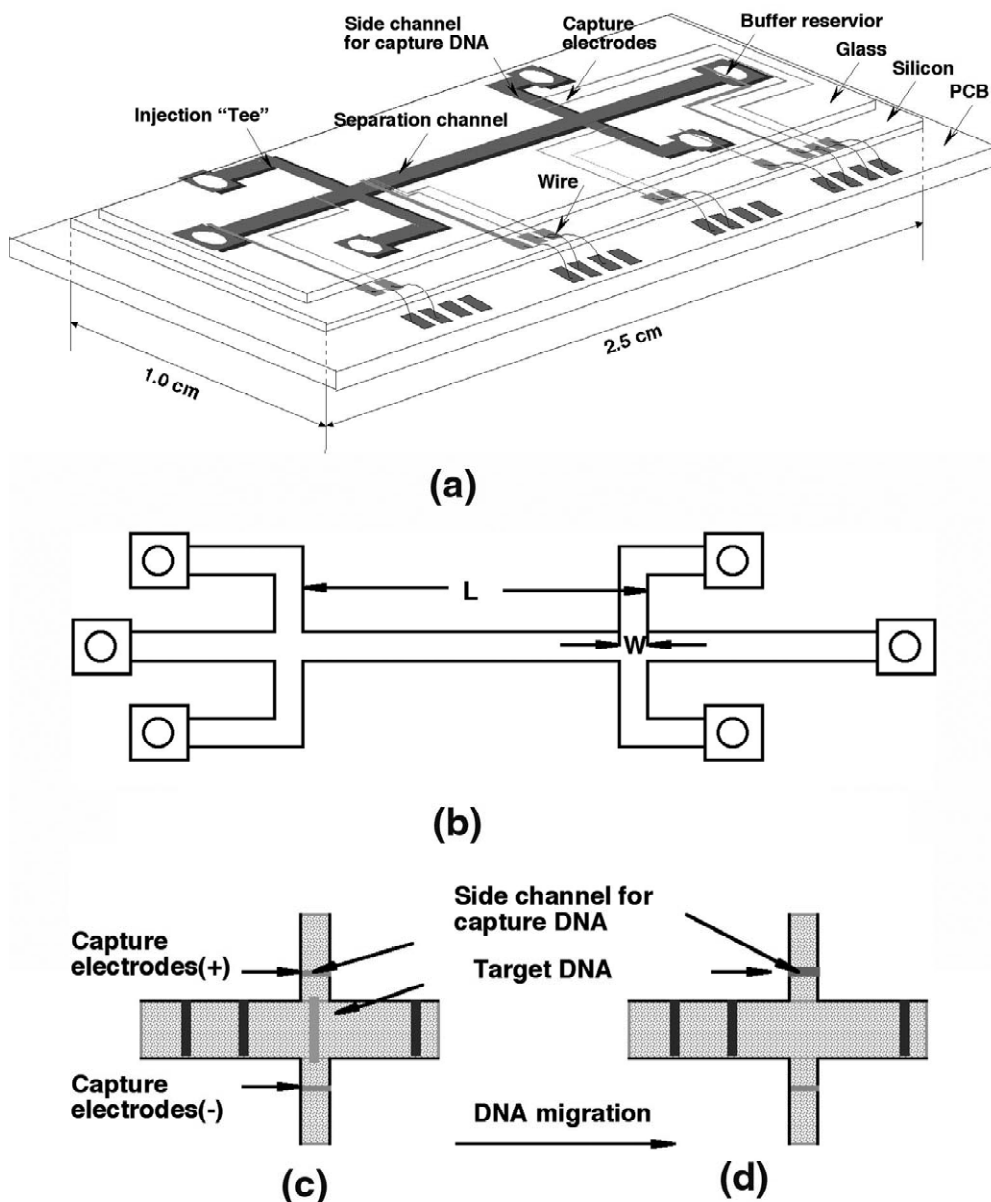


Fig. 2. (a) Schematic of the on-chip electro-elution device. In the orientation depicted in this photograph, DNA migration proceeds from left-to-right. (b) Top view of glass channels for separation and extraction. *L* is the downstream location of the side channel and *W* is the width of the side channel. (c) Schematic operation showing DNA bands migrating through the intersection formed by the separation and side channels. (d) Schematic operation showing the target DNA captured by the electrode in the side channel.

Electrophoresis and extraction experiments were performed using ReproGel (Amersham Pharmacia Biotech, Piscataway, NJ, USA), a commercially available photo-polymerized cross-linked poly-acrylamide gel. The gel solution was prepared by mixing 5 μl of solution acrylamide stock solution with 10 μl of the photoinitator solution, and is loaded into the entire channel network by placing a drop of premixed solution at one end of the separation channel. In some cases, Page Plus acrylamide

stock (Amresco, Solon, OH, USA) was substituted for the corresponding ReproGel reagents in order to allow the use of varying gel concentration. All reagents were used as received. The gels were cast using a previously described masking procedure, which allows a well-defined flat gel interface to be precisely positioned inside the electrophoresis channel [24]. An initial polymerization period of 3 min was used to set the gel interface, after which the interface mask and accompanying unpolymerized solution were removed. The masked region and reservoirs were then refilled with electrophoresis buffer and UV polymerization was allowed to continue for additional 5–10 min depending on the gel matrix used. This protocol enabled the most precise positioning of the gel interface within the microfabricated channel. Polymerization was performed using the ReproSet UV illumination source (Amersham Pharmacia Biotech) designed for use with the ReproGel cassettes in macroscale DNA sequencers, no additional surface treatment of the microchip glass channels was applied since the electric fields employed are below the range where electroosmostic flow significantly influences electrophoretic mobility.

2.4. Data analysis

After injecting the DNA sample into the gel, fluorescence from the migrating bands was detected using an Olympus SZX 12 fluorescence stereoscope with a mercury arc illumination source, and imaged using a Hamamatsu C2400-08 SIT camera (Hamamatsu Corp., Bridgewater, NJ, USA). The camera output was recorded, digitized and intensity profiles corresponding to the migrating bands were obtained by extracting the variation in fluorescence intensity with time at a fixed location in the gel using Transform two-dimensional image analysis software (Research Systems, Boulder, CO, USA) on an Apple PowerMacintosh (Cupertino, CA, USA) computer.

3. Results and discussion

3.1. Principle and design of band extraction

The technique we use to extract migrating bands in an electrophoresis gel is the addition of an electric field perpendicular to the separation direction. The perpendicular field is generated using on-chip electrodes, and the method of extraction is equivalent to conventional-scale electro-elution. Since the electrodes are fabricated directly in the channels, they can also be used to capture the eluting bands. The device fabricated for these studies consists of a separation channel and a sample injection ‘‘Tee’’ region for sample introduction (Fig. 2a). A side channel is positioned at approximately 0.5 cm distant from the sample introduction channel and perpendicular to the separation channel to capture and extract the target bands (Fig. 2b). When DNA fragments migrate through the separation channel under an applied electric field, they will separate into individual bands containing like-sized fragments. As the individual band of interest migrates into the intersection formed by the separation and side channel (Fig. 2c), an electric field perpendicular to the direction of separation field is applied and the target band then can be attracted to the capture electrode (Fig. 2d). The band of interest is isolated using an electric field perpendicular to the migration direction of the remaining bands, which causes it to migrate out of the gel and into free buffer.

In order to design the on-chip extraction module, the geometry at the intersection between the separation and side channels, including the width (W) and downstream position (L) of the intersection (Fig. 2b), has to be determined. The width (W) should be as wide as the target band in order to extract as much target DNA as possible. Conversely, there is also a maximum width based on the distance between migrating DNA bands beyond which neighboring bands will contaminate the target band during extraction. As the DNA band migrates, it also diffuses and disperses along the length of the channel. As a result, the DNA band becomes broader as it migrates forward. Therefore, DNA peak width and spacing with respect to downstream position must first be measured in order to determine these two parameters.

The peak width is typically measured at half height (full width at half maximum, FWHM or W_h). For a Gaussian peak, the width at half maximum is related to the variance of the spatial concentration distribution governed by diffusion-convection equation [25]:

$$W_h = \sigma_{tot}\sqrt{[2 \ln(2)]} \tag{1}$$

The total variance σ_{tot} is the sum of the individual contributions of different factors (presumably independent) including injection plug length σ_{inj} and dispersion [26], which can be expressed as:

$$\sigma_{tot}^2 = \sigma_{inj}^2 + 2D^E t \tag{2}$$

where D^E is the longitudinal dispersion coefficient of the DNA fragments in the gel, t is the migration time. The migration time can be expressed as $t = L/(\mu \cdot E)$, where L is the separation length, E is the electric field and μ is mobility of a migrating DNA fragment.

Since the migrating bands can be detected at any desired location in a microfabricated electrophoresis channel, we are able to determine the peak width of each migrating band by moving the observation window at a fixed electric field [21]. Peak width measurements in a 100 bp double-stranded DNA ladder are very similar across the size range of the sample (100–300 bp) shown in Fig. 3a. At a separation length of 5 mm, the peak width is around 100–130 μm. The peak spacing between neighboring bands increases linearly with separation length (Fig. 3b). At a separation length of 5 mm, the spacing between the 100 and 200 bp bands is around 800 μm. These two plots provide the basis for predicting the peak width and spacing at any downstream distance (L), so that the side channel can be properly sized and positioned. Based on these measurements, we use a 200 μm wide side channel, located 5 mm downstream from the sample injection "Tee".

The dispersion coefficient is a key parameter to characterize the peak broadening during electrophoresis. By linearly fitting the square of the variance with respect to migration time, the dispersion coefficient can be calculated based on Eq. (2). The dispersion coefficient ranges from $1.07 \cdot 10^{-7}$ (100 bp) to $6.6 \cdot 10^{-8}$ cm^2/s (300 bp), which agrees well with literature values [27]. This data can be used for predicting the peak width under the similar conditions.

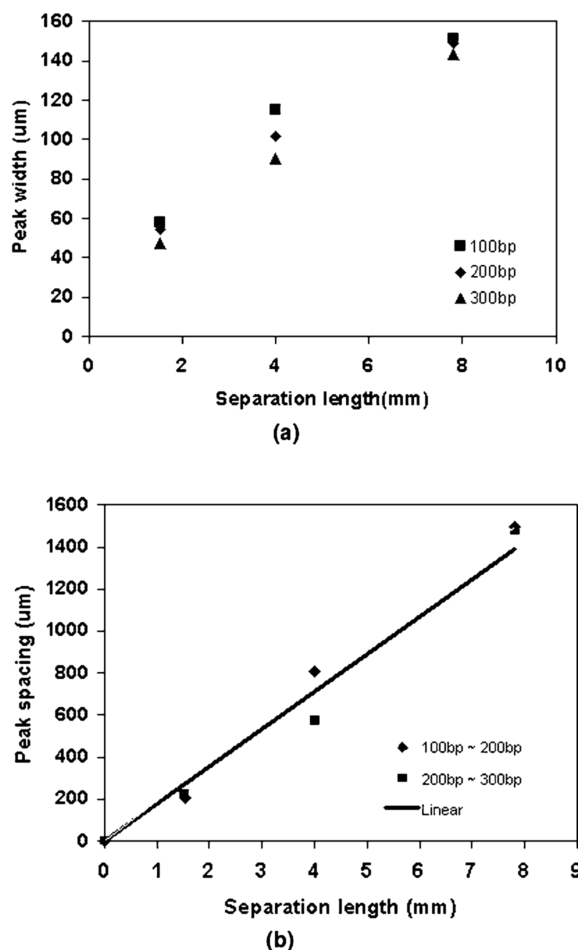


Fig. 3. (a) Peak width and (b) peak spacing for separation of a 100-bp ladder in crosslinked polyacrylamide (8% T) [T=(g acrylamide + g N,N'-methylenebisacrylamide)/100 ml solution]. Detection windows are located at distances of 1.5, 4.0 and 7.8 mm downstream from the gel interface (E=30 V/cm).

3.2. Selective extraction of DNA fragment

The perpendicular side channel used to selectively extract the desired bands modifies the electric field in the separation channel and may adversely affect the separation performance. Since extraction of DNA fragments depends heavily on how well individual bands are separated, the separation performance of the device was carefully investigated both theoretically and experimentally.

The electric field is governed by Poisson equation:

$$\varepsilon \nabla^2 \phi = -\rho_e \tag{3}$$

where ε is the dielectric constant, ρ_e is the charge density of ions and ϕ is the electrical potential. For

electrophoresis in microchannels, we can assume steady electric field, uniform fluid density and a uniformly charged solid surface. Furthermore, the Debye layer thickness is small compared to any channel dimension. Under these restrictions, the electric field potential outside the Debye layer is governed by the Laplace equation [28]:

$$\nabla^2 \phi = \frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} = 0 \quad (4)$$

This equation can be used to predict the shape of the electric field in our device.

Based on two-dimensional simulation of electric field, we find the electric field streamline will expand into the side channel (Fig. 4a). This result implies the DNA band will partially migrate into the side channel as it arrives at the intersection, regardless whether electric fields are generated in the side channel. This is in a good agreement with experimental results (Fig. 5a). In addition to the expansion, the electric field strength is reduced symmetrically near the side channel (Fig. 4b). This decrease results in a slowing down of the two ends of the DNA band passing near the side channel and can therefore increase the band broadening.

The broadening observed near the side channel increases the possibility of neighboring bands contaminating the DNA of interest. In order to investigate the occurrence of this phenomenon, a detection window is positioned right below the intersection of the side channel and the intensity data is recorded as an undesired band migrates by (Fig. 5a,b). A peak in intensity occurs as the first fragment passes by, followed by a return back to baseline before the second band reaches the intersection, indicating there is little residue left over from the first fragment. It appears that the designed side channel has little effect on separation of DNA fragments.

After the undesired band passes by the side channel, the next step is extraction of the target fragment. As the target band arrives at the intersection, the main electric field is switched off and a transverse electric field is activated to capture the band. Fig. 6a shows this procedure for a 100-bp ladder sample. In this experiment, the second band in a series of bands is separated from a mixture and selectively captured on a side channel electrode (Fig. 6b).

During these experiments, we observed some portions of the extracted band lagging behind during extraction. Simulation results show that the electric field streamlines expand into the main channel as the side channel capture electrode is activated (Fig. 6c). Similarly, the expanded electric field has a smaller magnitude along the main channel and consequently results in deceleration of some portions of the extracted band. Since extraction of pure target DNA is of primary importance at this point, the residue left behind does not pose significant concern. However, capturing the entire band could be necessary in order to obtain a sufficient amount of DNA for subsequent analyses. Extraction of the entire band can by achieved by increasing the width (W) of the side channel with the caveat that the spacing between bands would have to be larger (i.e. longer separation distance).

3.3. Multiple DNA fragments extraction

While we have successfully demonstrated the feasibility of an on-chip electro-elution module to selectively extract individual DNA fragments, the ability to extract multiple fragments is also important for a variety of biological assays. Different strategies can be employed in achieving this goal, such as extracting target fragments in parallel by positioning multiple side channels across the separation lane, or electro-eluting individual fragments at the end of the separation lane and collecting them in series. Since microelectrodes can be readily positioned at any location on the silicon substrate, it is possible that multiple fragments can be extracted by employing a series of electrodes in a single side channel.

The effectiveness of this approach depends on the ability of the electrophoresis gel to resolve DNA fragments. To fully exploit the benefits of separation in a miniaturized format, we are desire to achieving extraction of well-resolved fragments in a short distance. To demonstrate separation performance, a 100-bp ladder sample was loaded into a gel by applying an electric field of 30 V/cm for approximately 10 s (Fig. 7, I). After electrophoresis at 27 V/cm for approximately 5 min, all the DNA peaks can be resolved over a distance of 5 mm (Fig. 7, II). This result shows crosslinked polyacrylamide gel offers excellent separation performance over a

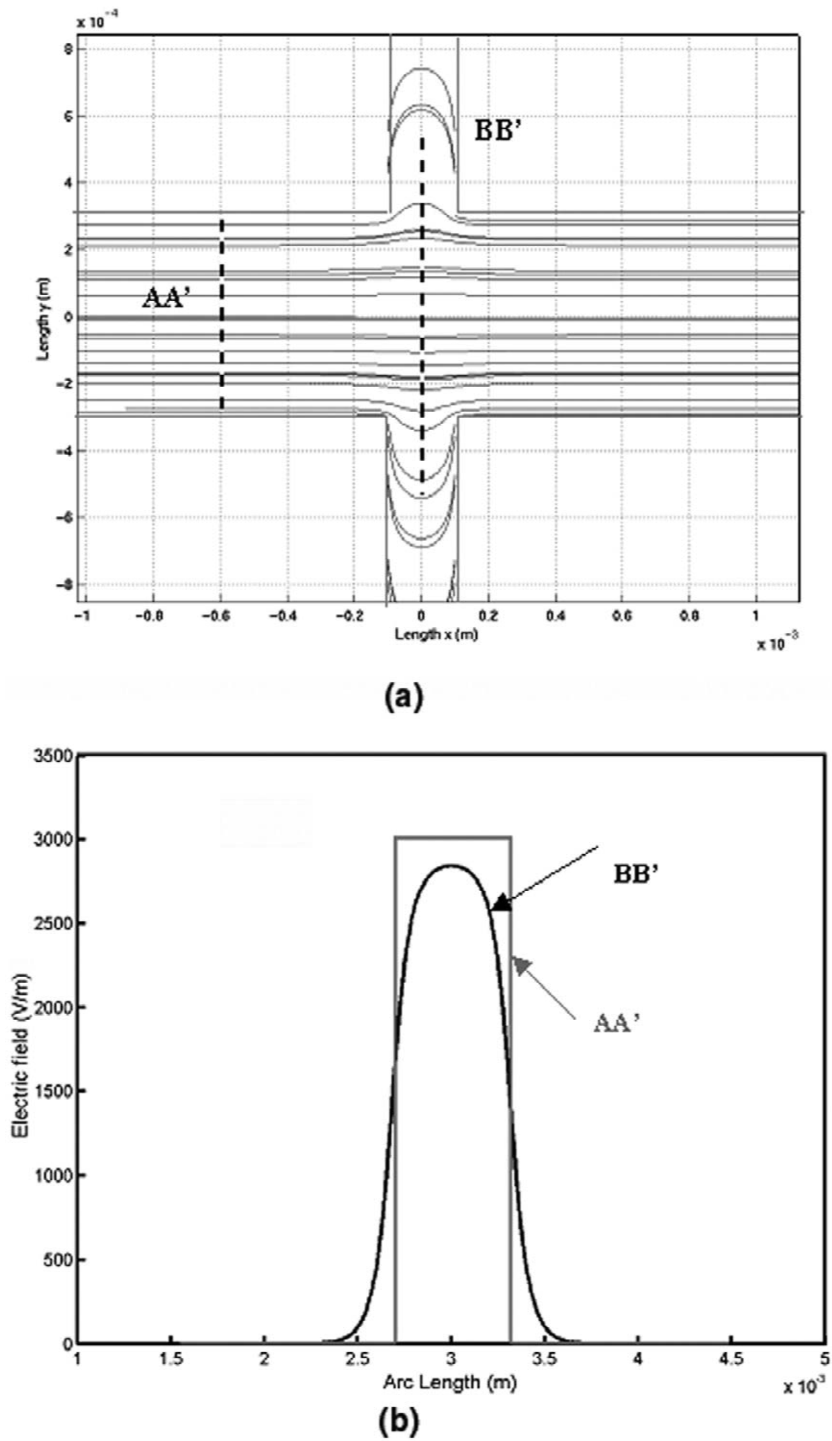


Fig. 4. (a) Electric field simulation at the intersection showing electric field expansion (main channel: 1 cm×600 μm, side channel: 0.3 cm×200 μm, potential spanning the main channel: 30 V) Solid line is electric field streamline. (b) Electric field distribution at the cross-section of AA′ and BB′.

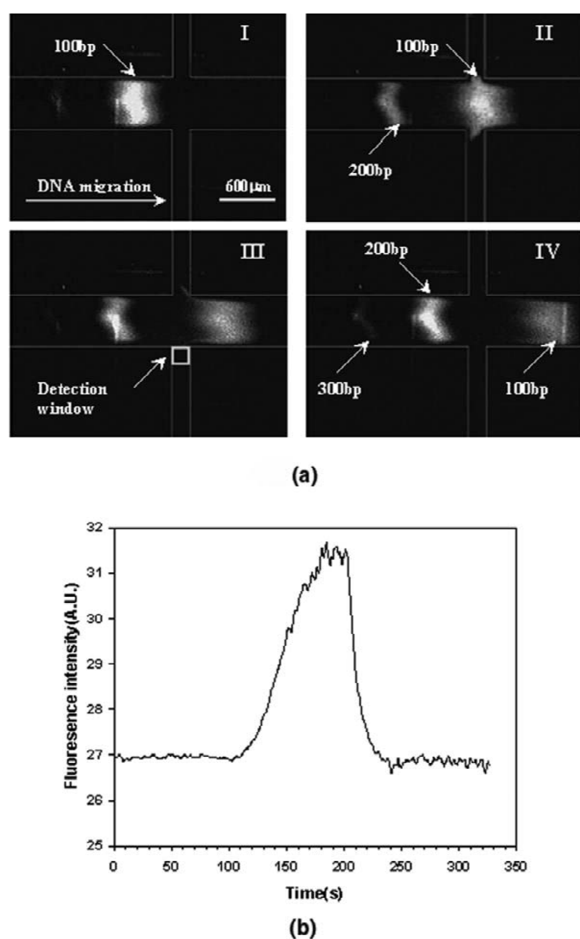


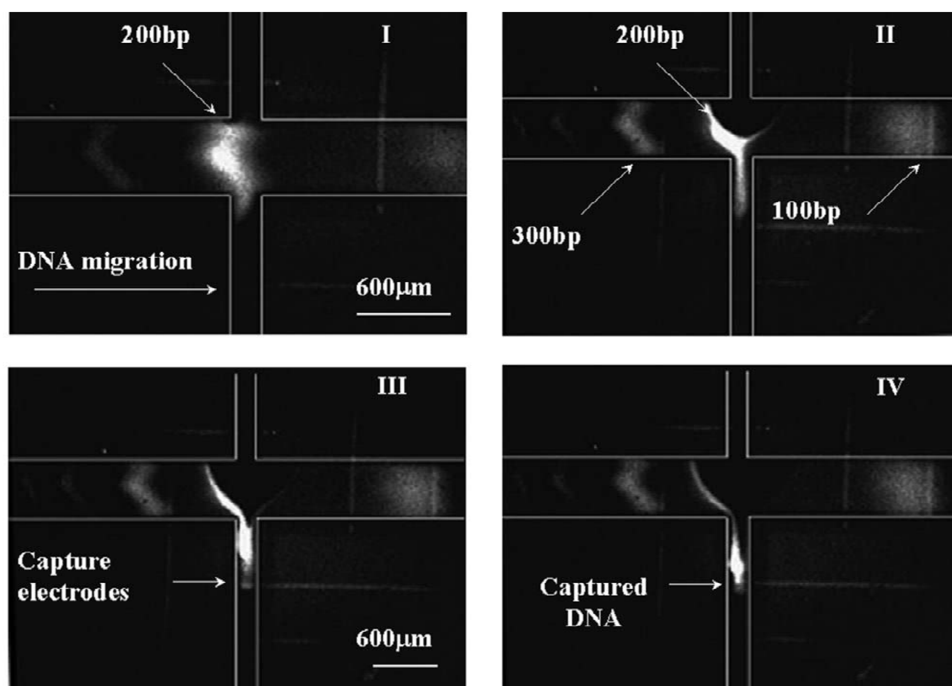
Fig. 5. (a) Video sequences depicting the first migrating DNA fragment (100 bp) passing by the intersection during separation of a 100 bp ladder in crosslinked polyacrylamide (8% T, $E=30$ V/cm). The side channel is positioned 5 mm downstream from the gel interface. Pictures are shown at (I) 1, (II) 3, (III) 4, and (IV) 5 min after the extraction begins. (b) Fluorescence intensity plot with detection window in the side channel indicating no residue is left due to expansion of the first fragment.

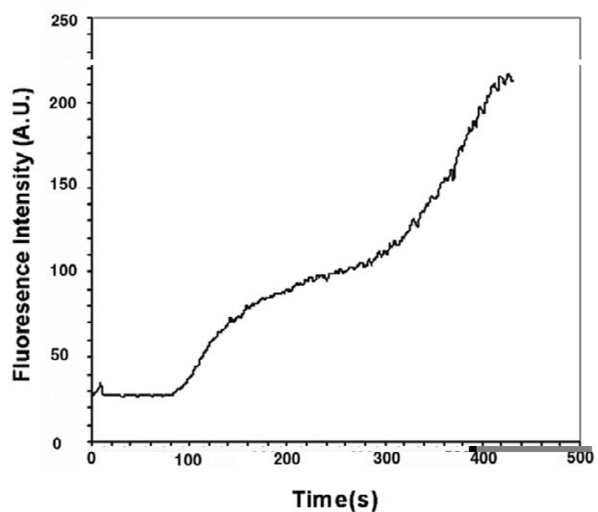
short distance, which is in agreement with the measurement of dispersion in crosslinked polyacrylamide gel matrix [21] and comparison with linear polyacrylamide typically used in CE [22].

By activating capture electrodes spanning the side channel when the first band arrives at the intersection, a transverse electric field of 23 V/cm is then turned on and the entire target DNA band is attracted to the anode and temporarily immobilized on that electrode (Fig. 7, III, IV). For these conditions, we are able to extract the entire band with minimal leakage from the successive fragment. After the first band is captured successfully, the electric field across the main channel is turned on again and the remaining nine bands continue migrating through the gel. Once the second fragment migrates into the intersection, the electric field across the side the channel is switched on and the second fragment is captured at a different electrode than the first band (Fig. 7, V,VI). To prevent the first DNA fragment from being released, an identical potential is applied to the first capture electrode. By this means, two different fragments can be captured at individual electrodes.

Employing a series of microelectrodes in a side channel is not the only means to extract multiple fragments. Fragments can be collected at the exit of the electrophoresis column by driving individual band into different zones [18]. It is beneficial in terms of speed since high voltage is applied and controlled automatically by in-house built software. However, this strategy usually leads to considerable dilution of the sample especially when external electrodes are applied and therefore not is favorable for further downstream sample processing. The accumulation of the target fragments on microelectrodes concentrates instead of dilutes the sample and is beneficial for subsequent analyses. The fragment of interest can be readily transported from one location to another on the chip by electrostatic force using these microelectrodes.

Although we were able to successfully capture two individual bands in this device and demonstrated the potential for system integration, there are still other issues that need to be properly addressed to further improve the performance. For instance, we observed the remaining eight bands disperse heavily while capturing the second band. The remaining bands are subjected to not only diffusional band broadening but also dispersion, which impair the extraction by increasing the possibility of carry-over contamination from the non-selected fragment. Rapid separation by applying high electric field can alleviate this problem. Even though a side channel can be positioned at a longer downstream distance to obtain large band spacing in order to reduce carry-over contamination, it compromises the merits of miniaturization and band broadening due to dispersion has to be addressed properly.





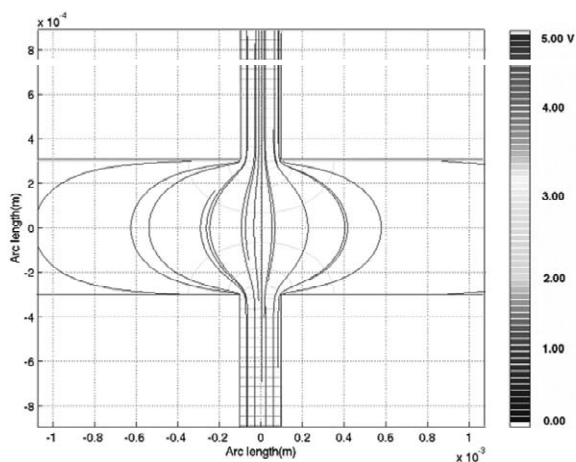


Fig. 6. (a) Video sequences depicting the extraction of the second migrating DNA fragment (200 bp) during separation of a 100-bp ladder in crosslinked polyacrylamide (8% T, $E=30$ V/cm). Capture electric field is 31 V/cm. Pictures are shown at (I) 0, (II) 100, (III) 200, and (IV) 400 s after the extraction begins. (b) Fluorescence intensity plot showing accumulation of the target DNA on the capture electrode. (c) Electric field simulation at the intersection showing electric field expansion (main channel: 1 cm×600 μm, side channel: 0.3 cm×200 μm, potential spanning the side channel: 5 V). Solid line is electric field streamline.

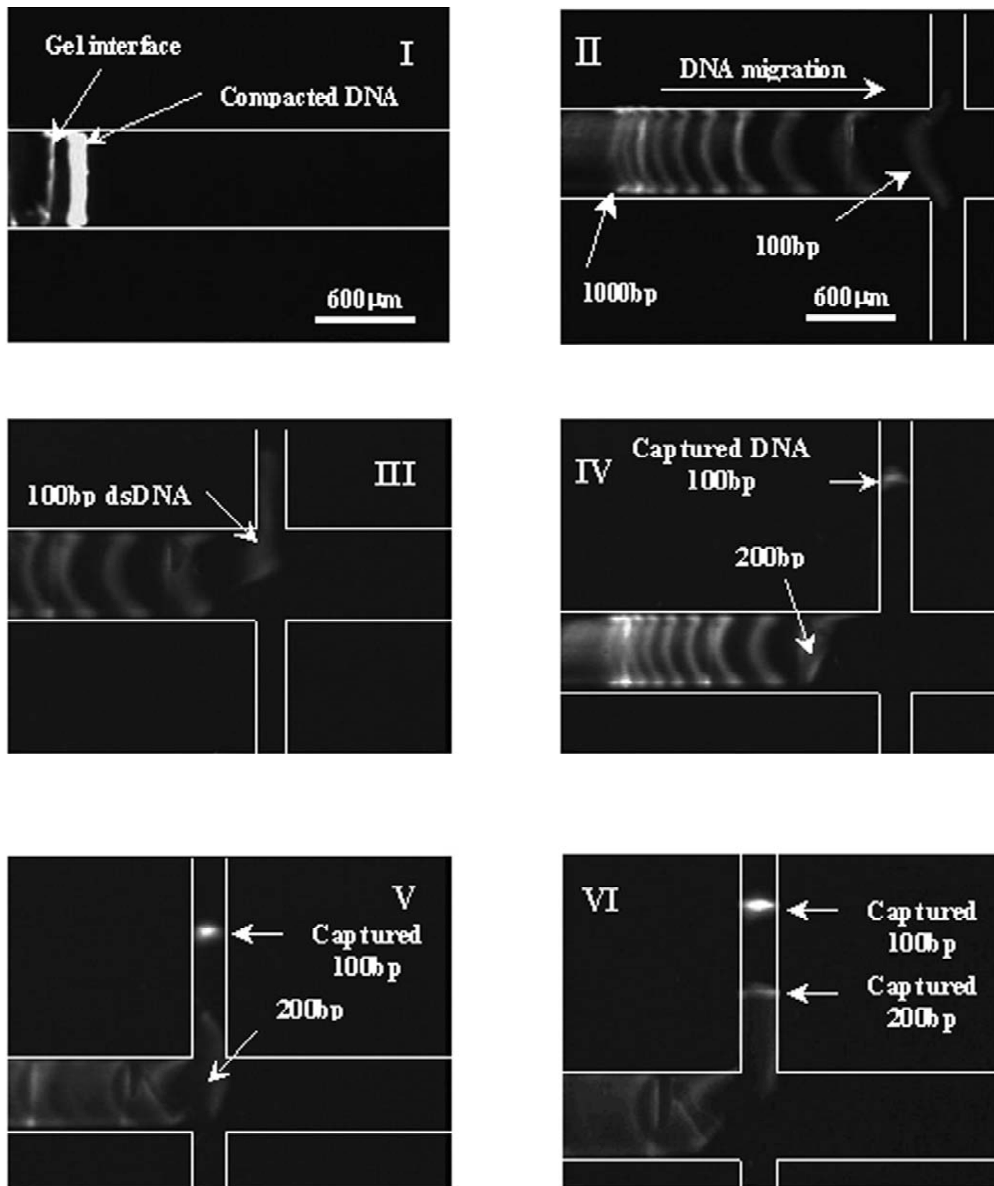


Fig. 7. Video sequences depicting multiple bands extraction during separation of a 100-bp ladder in crosslinked polyacrylamide(5% *T*, $E=30$ V/cm) (I) sample compaction, (II) separation, (III,IV) the extraction performance of first migrating DNA fragment (100 bp) at a electric field of 27 V/cm. (V,VI) The extraction of the second DNA fragment (200 bp) at a electric field of 31 V/cm.

An advantage of using microfabricated electrodes is not only that they can be used to capture DNA fragments but also that they can be used to ‘‘shape’’ the electric field. For example, by positioning microelectrode arrays along the edges of the intersection (Fig. 8a,b), we can confine the target band and

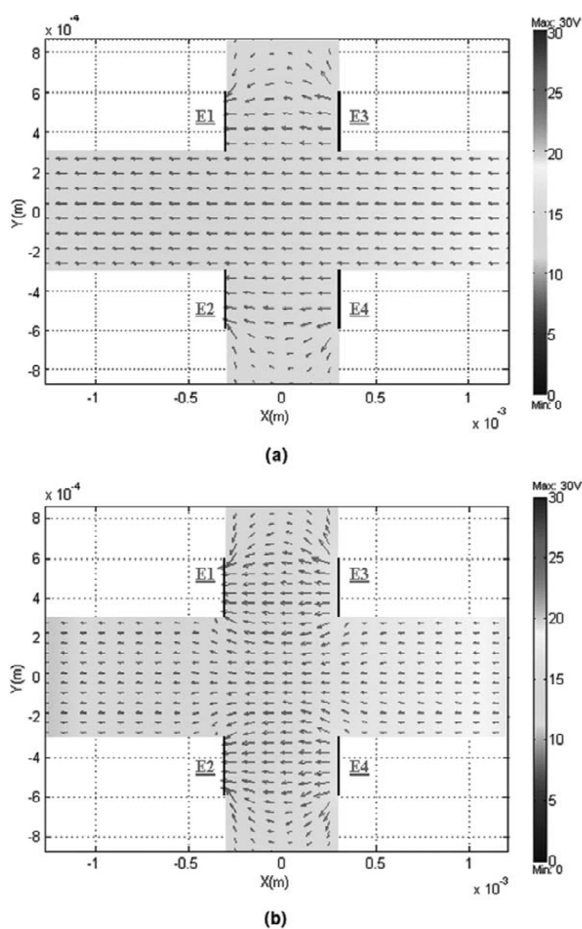


Fig. 8. (a) Shaped electric field distribution at the intersection to reduce expansion (Main channel: 1 cm×600 μm, side channel: 0.3 cm×600 μm, potential spanning the main channel: 30 V, $E1=E2=14.1$ V, $E3=E4=15.9$ V) Arrow is electric field streamline. (b) Shaped electric field distribution at the intersection to confine the target DNA band (Main channel: 1 cm×600 μm, side channel: 0.3 cm×600 μm, potential spanning the main channel: 30 V, $E1=E2=13$ V, $E3=E4=17$ V). Arrow is electric field streamline.

extracted it using relatively wider side channel with less carry-over contamination. Parallel side channels can also be readily added onto the original design to extract multiple fragments simultaneously. Other changes, such as using polymer substrates [29] and thermo-reversible gel matrixes would improve the economic potentials of these devices [30]. Further refinement of the system may be realized through theoretical analysis and rigorous experimentation of DNA migration behavior in these multiple channels network under electric fields of various shapes.

4. Conclusions

We have demonstrated the feasibility of on-chip electro-elution to selectively extract single or multiple DNA fragments during electrophoresis using a crosslinked polyacrylamide matrix. The microfabricated device for band extraction presented in this work offers several advantages. First, it is a simple device that utilizes microelectrodes to extract DNA molecules. Compared to traditional methods, this technique reduces the personnel and time required to perform manual extraction of DNA fragments from gel plates, and eliminates the need for complex mechanical systems required for collecting fragments. Second, this device can be readily integrated into complex DNA analysis devices that involve several other steps such as restriction digestion or PCR. Third, by employing a cross linked polyacrylamide gel matrix, the extraction can be achieved over very short distances at a relatively low electric fields, making this on-chip extraction module suitable for battery powered operation for portable analysis application.

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