Microfabrication Technology for the Production of Capillary Array Electrophoresis Chips

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Abstract. **Improvements in the fabrication, sample handling and electrical addressing of capillary array electrophoresis (CAE) chips have permitted the development of high density, high-throughput devices capable of analyzing 48 samples in about 20 minutes. The fabrication of high density capillary arrays on 10 cm diameter substrates required the characterization of glasses that yield high quality etches and the development of improved sacrificial etch masks. Using these improved fabrication techniques, high-quality, deep channel etches are routinely obtained. Methods for bonding large area substrates and for drilling arrays of 100 or more access holes have also been developed. For easier sample introduction, we use an array of sample wells fabricated from an elastomeric sheet. The practicality of these technologies is demonstrated through the analysis of 12 DNA samples in parallel on a microfabricated CAE chip, the development of methods for injecting multiple samples onto a single capillary without cross contamination, and the operation of a microfabricated array of 12 capillaries with 4 sample injections per capillary that can analyze 48 samples.**

Key Words. **DNA Analysis, capillary array electrophoresis, confocal fluorescence detection, multiplex sample injection, glass micromachining, capillary electrophoresis chips**

In the last five years it has been shown that capillary electrophoresis (CE) systems can be photolithographically fabricated on planar glass sandwich structures and used to perform high-quality and high-speed electrophoretic separations. CE chips have been used to separate fluorescent dyes [1,2], fluorescently labeled amino acids [3–5], metal ion complexes [6], and blood serum cortisol [7] as well as DNA samples such as DNA restriction fragments [8,9], PCR products [8], short oligonucleotides [10] and sequencing fragments [11]. Intersecting injection and electrophoresis channels reduce the width of the injection plug to approximately that of the injection channel itself. Narrow plug widths reduce the separation time because a shorter electrophoresis length is required to achieve the desired resolution. A significant potential advantage of microfabricated capillaries is that dense, complex channel patterns and interconnects can be easily defined.

The microfabrication of integrated DNA analysis systems promises to add functionality and speed that would be impossible with conventional sample preparation and CE analysis techniques. Microfabrication is a planar technology that allows for integration of features such as temperature control [12,14], fluidic components [9,13], and optics [15] onto a single substrate. We have recently demonstrated the functional integration of PCR and CE in a microfabricated system [14]. Moreover, since microfabrication is scaleable, it should be possible to produce highly-parallel, high-density arrays to yield extremely high throughput systems.

As in capillary array electrophoresis (CAE) [16] individual capillaries can be fabricated in a parallel array to increase throughput [17]. In order to fabricate functional capillary arrays and other integrated systems while making their operation feasible and practical, improvements in fabrication methods, detection methods and sample introduction and loading methods are needed. A primary goal of this manuscript is to describe some of the improved methods and approaches we have developed to address these issues.

Fabrication Technology

Most CE chips developed at Berkeley [8,11,14,17] and elsewhere [1,3] have used shallow (8–12 μm) channels. We have previously fabricated shallow channels on soda lime microscope slides (50 × 75 × 1 mm, catalog no. 12-550C, Fisher Scientific) using hard-baked photoresist (Shipley, 1818) to define the etch pattern. Because soda lime glass exhibits high etch rates, less aggressive etchants, such as buffered hydrofluoric acid can be used and photoresist alone will provide sufficient

protection as an etch mask for shallow etches. Moreover, many defects and impurities in the glass do not influence the pattern quality with shallow etches. This approach has the advantage of cheap, easy and rapid fabrication. In some applications the high resistance to fluid flow of these shallow channels is beneficial. For example, in the integration of PCR and CE, the shallow channels filled with sieving matrix were used as an ''electrophoretic valve'' between the two chips because the viscous sieving matrix prevents flow of PCR mixture during thermal cycling [14].

However, fabrication of deeper channels has a number of important practical advantages. Deeper channels facilitate filling of CAE chips with viscous separation matrices, such as 0.75% hydroxyethylcellulose (HEC), and improve the ability to flush the matrix out after use. Another benefit is an increased path length for optical detection that yields a linear increase in signal. However, deep channel etching requires more robust sacrificial layers with improved etch resistance. Additionally, fabricating capillary array systems requires large areas (up to 10 cm diameter wafers) to be essentially free of defects. Because of the isotropic nature of the wet chemical etch, a single defect such as a scratch, dust particle or pin hole in the protective masks before etching becomes a > 20 μm wide pit that could render a high density array device useless.

Sacrificial etch masks

To etch deep channels (> 15 μm) with smooth sidewalls, higher quality glasses and aggressive etchants are required. Hard baked photoresist no longer provides sufficient protection because the etchant attacks the interface of the photoresist and the glass and can penetrate the protective mask; thus, a more resistant sacrificial layer is required. To achieve high quality etches of deep channels over 10 cm diameter substrates we have characterized the etch properties of glass using both Cr/Au and amorphous silicon as sacrificial etch masks.

Cr/Au has been used as a sacrificial etch mask by many groups [2,7,18,19] and is effective for many types of etchants. In these studies, we deposited a 100 Å Cr adhesion layer followed by a 2,000 Å gold protective layer onto clean substrates. The thick gold films act to reduce the number of pin holes. The substrates are then patterned with photoresist and the pattern is transferred to the sacrificial layer using aqueous Au etch (Gold Etchant TFA, Transene Company, Towley, MA) and Cr etch (Cr-7 Chromium Photomask Etchant, Cyantek Corp, Fremont, CA) solutions. The substrates with the photoresist/Au/Cr etch masks can be etched in a variety of HF based etchants optimized for the particular type of glass being used. Cr/Au etch masks are particularly useful with HF/HNO_3 etchants which attack other etch masks, such as amorphous silicon.

Although Cr/Au etch masks hold up well in aggressive etchants, the etchant will eventually penetrate through the protective layers and attack the glass, producing pitting on the surface. Using Borofloat as a substrate, the Cr/Au masks can withstand ~ 5 minutes in 49% HF to yield an etch depth of 35 μm before showing a significant number of defects. Additionally, Cr/Au masks exhibit defects that are associated with stresses within the metal layers. Figure 1A shows an example of this type of defect, which appears as a wedge shaped notch etched into the sides of the array. This is probably due to small defects present at the edge of the mask; due to internal stresses, the metals peel up causing preferential etching. Such defects are also seen with thick (> 1700 Å) Cr etch masks [20]. A single defect of this type can destroy an array of channels since the defects are roughly perpendicular to the channel. A characteristic defect that is present in all etch masks but is particularly problematic in Cr and Cr/Au masks is pinholes. Since the deposition is often done in a relatively dirty evaporation system it is difficult to keep the surfaces completely free of dust and chips of metal. Although photoresist often covers the pinholes, the etchant can quickly get through the photoresist alone. Figure 1B details the features that are observed when a Borofloat substrate with a pin hole in the Cr/Au etch mask is etched with HF. The depth of the circular defect is 25 μm which is equivalent to the depth of the channel. Smaller etched pits due to pin holes that have opened up later in the etch are observed near the larger defect.

As an etch mask, amorphous silicon [21] has shown the best resistance to HF etching and the fewest defects. We have deposited amorphous silicon films using a table top Plasma Enhanced Chemical Vapor Deposition (PECVD) reactor (PEII-A, Technics West Inc, San Jose, CA). Silicon is particularly resistant to HF etches and when used as an etch mask, can withstand etches up to 70 μm deep [20]. However, the depth possible with amorphous silicon as the etch mask is highly dependent on the quality of the deposition and the etchant being used. Figure 2 presents an electron micrograph of features etched by 49% HF with amorphous silicon as an etch mask. The image is a corner of a plateau where the area below and to the right has been etched to a depth of 25 μm. The smooth sidewalls and absence of defects on both the etched and unetched surfaces demonstrates the quality of etch that can be achieved. One of the advantages of using amorphous silicon as an etch mask is that the substrates can go through a final cleaning step in the deposition chamber before the actual deposition. After a piranha clean (conc. H_2SO_4 and H_2O_2, 2:1) the substrates are placed in the PECVD chamber that has

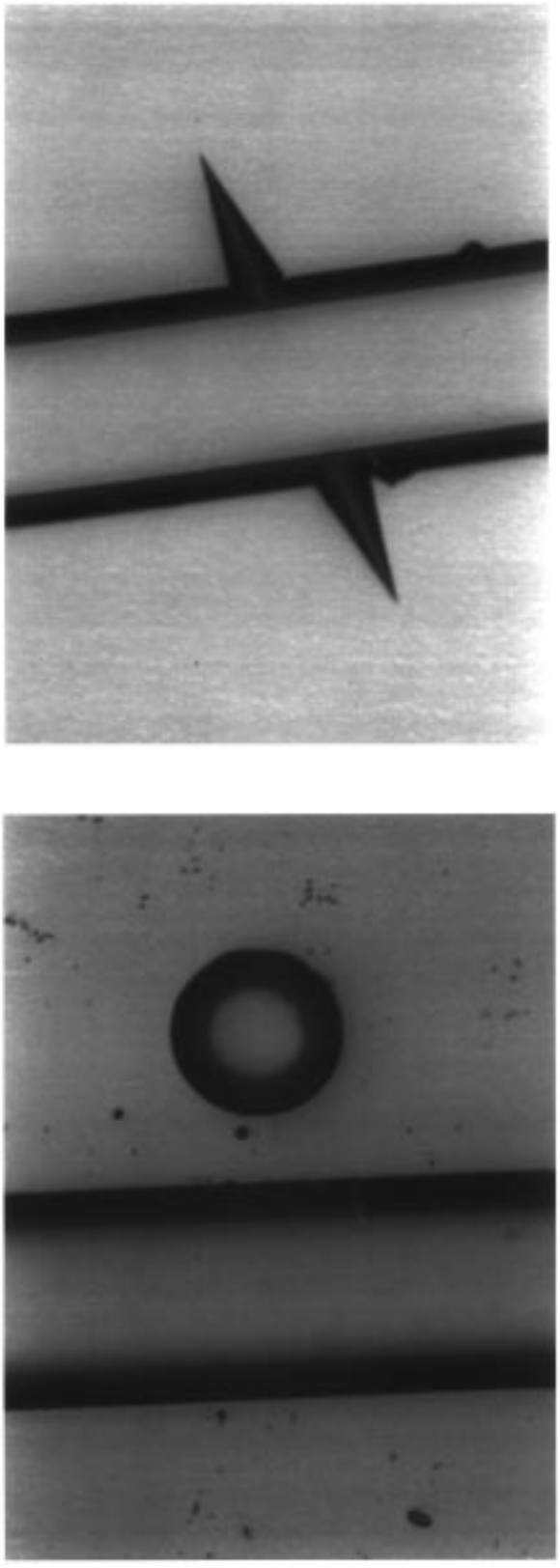


Fig. 1. *(A) Optical image of a defect in Borofloat glass etched to a depth of 25 μm using 49% HF and Cr/Au (150 Å/1,500 Å) as the etch mask. This defect is characteristic of thick Cr and Cr/Au sacrificial etch masks. (B) Optical image of a defect in a Borofloat substrate caused by a pinhole in the Cr/Au mask after an etch with 49% HF to a depth of 25 μm.*

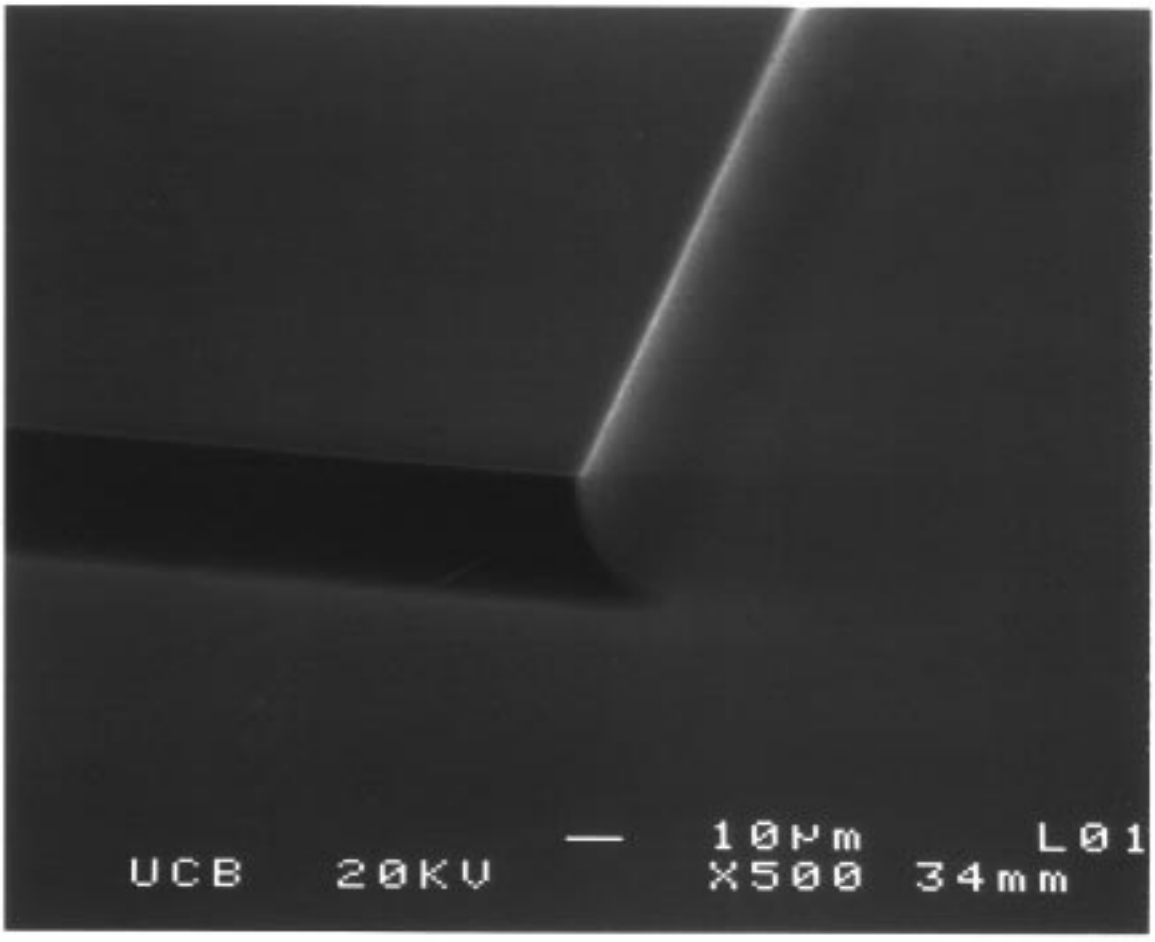


Fig. 2. Scanning electron micrograph showing a corner of two crossed channels etched in Borofloat. The areas to the right and bottom of the image have been etched 25 μm deep while the area to the upper left was protected by a 1,500 Å thick amorphous silicon sacrificial etch mask.

been precoated with amorphous silicon. Precoating the chamber traps any impurities underneath the capping layer enabling higher quality depositions. Before the actual deposition, a 60 s O_2 plasma ash is performed to clean off any stray dust particles. The deposition can then be performed without exposing the substrates to the ambient atmosphere. Photoresist (Shipley, 1818) is spun at 5,000 rpm onto the amorphous silicon coated substrates. After exposing the substrates with a pattern on a Quintel contact printer and developing in Shipley Developer concentrate (1:1 with DI H_2O) the pattern is transferred into the amorphous silicon using a CF4 plasma etch in the Technics PECVD reactor.

High quality, thin (1000 Å) sacrificial layers of amorphous silicon have permitted etching of surfaces with smooth sidewalls to depths of 70 μm [20]; however, thicker layers (>1700 Å) can cause striations perpendicular to the channels. This is probably due to stresses at the edge of the etched channel from the overhanging sacrificial mask. These striations are much more pronounced when using Cr/Au as the etch mask. Figure 3 shows the intersection of two channels where these characteristic striations are visible on the side walls. The limiting factor for depth is the eventual penetration of the etchant through the etch mask, causing pitting on the surface of the glass. High quality amorphous silicon will hold up about twice as long as Cr/Au, making it possible to etch twice as deep.

Other defects

While many defects can be eliminated by using appropriate etch masks, others are inherent to the type of glass or the condition of the surface at the time of the etch. Glass which appears to be free of defects can still have localized stresses or microscratches due to polishing or simple handling errors during manufacturing. The stresses within the surface layers of the glass cause preferential etching and poor adhesion of etch

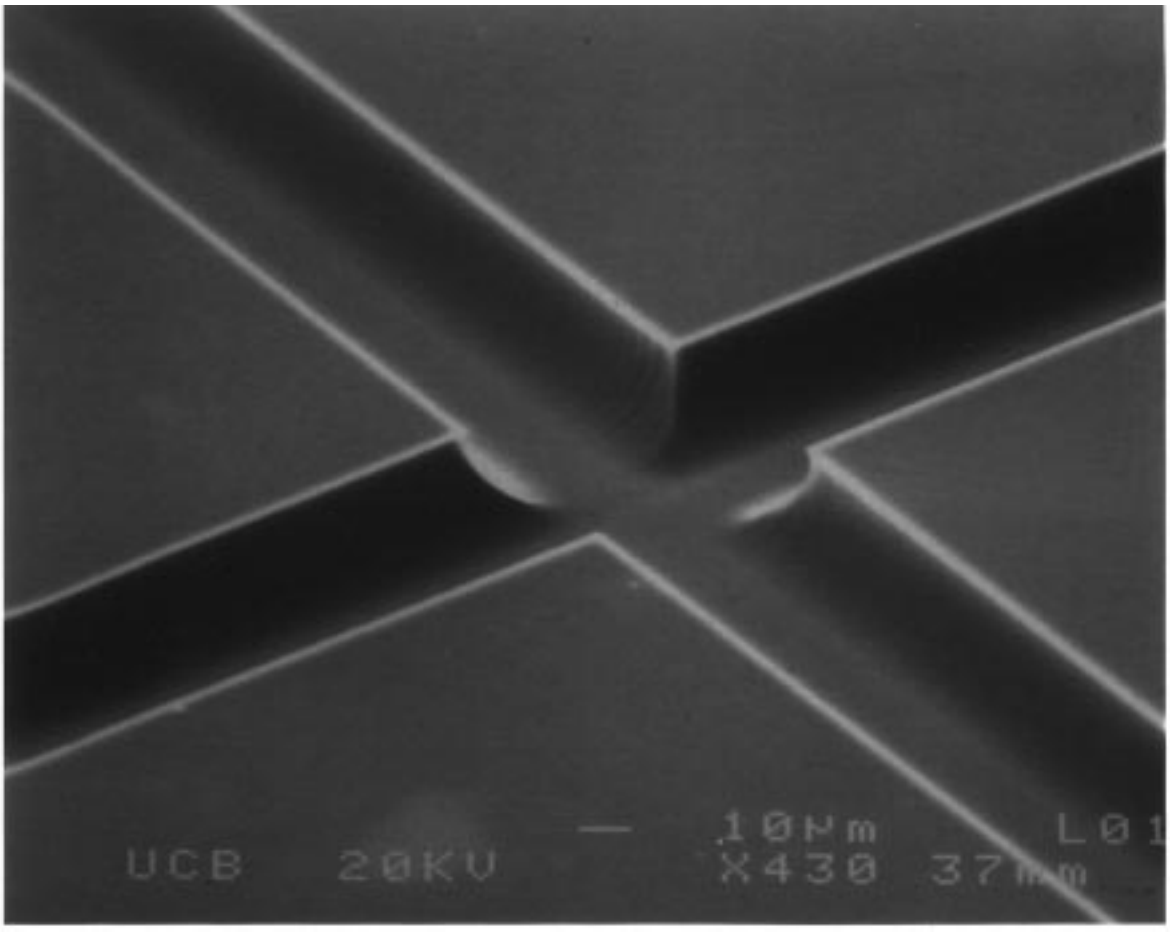


Fig. 3. *Scanning electron micrograph of the intersection of two 25 μm deep channels etched in Borofloat using a 1,700 Å thick amorphous silicon etch mask. Striations on the side walls of the capillary are evident near the intersection.*

masks along the scratch. Figure 4 shows a 7 μm deep etch of polished Borofloat, where the defects are evident on the etched surface and along the channel sidewalls.

One way to eliminate these problems is to anneal polished glass before processing [19]. This alleviates the stresses and gives high quality etches. However, annealing can alter the etch properties of some glasses and cause roughened etch surfaces. Many glasses do not require polishing to obtain appropriate thicknesses or sufficiently smooth and flat surfaces for processing. However, even glasses which do not require polishing are susceptible to scratches caused by handling and cutting the glass into wafer sized substrates. For example, the array of capillaries shown in Figure 5A is unusable because of the preferential etching along one such scratch. Scratch defects have been eliminated by pre-etching the glass before deposition of the protective etch mask. The substrates are etched long enough to remove a few microns of bulk glass off the surface. This eliminates the stresses and although the scratches are etched preferentially during the pre-etch, they no longer inhibit the adhesion of the protective masks. Figure 5B shows the effect of pre-etching on a surface scratch. Because of the pre-etch, this minor defect will not affect the bonding of this device. Using these techniques, much higher success rates (up to 70%) for fabrication of deep channel capillary array electrophoresis chips have been obtained.

Glass etching. A high quality protective etch mask is critical for fabrication of deep channel array systems. The type of glass and the composition of etchant used to etch the glass are also very important. After characterizing several types of glass as possible substrates for CE chips [20], we have concluded that the glass that has the best etch quality and simplest processing is Schott Borofloat.

Borofloat is a borosilicate glass that is manufactured using the float process resulting in a glass that is highly uniform in thickness and composition, with a smooth and flat surface that does not require any mechanical

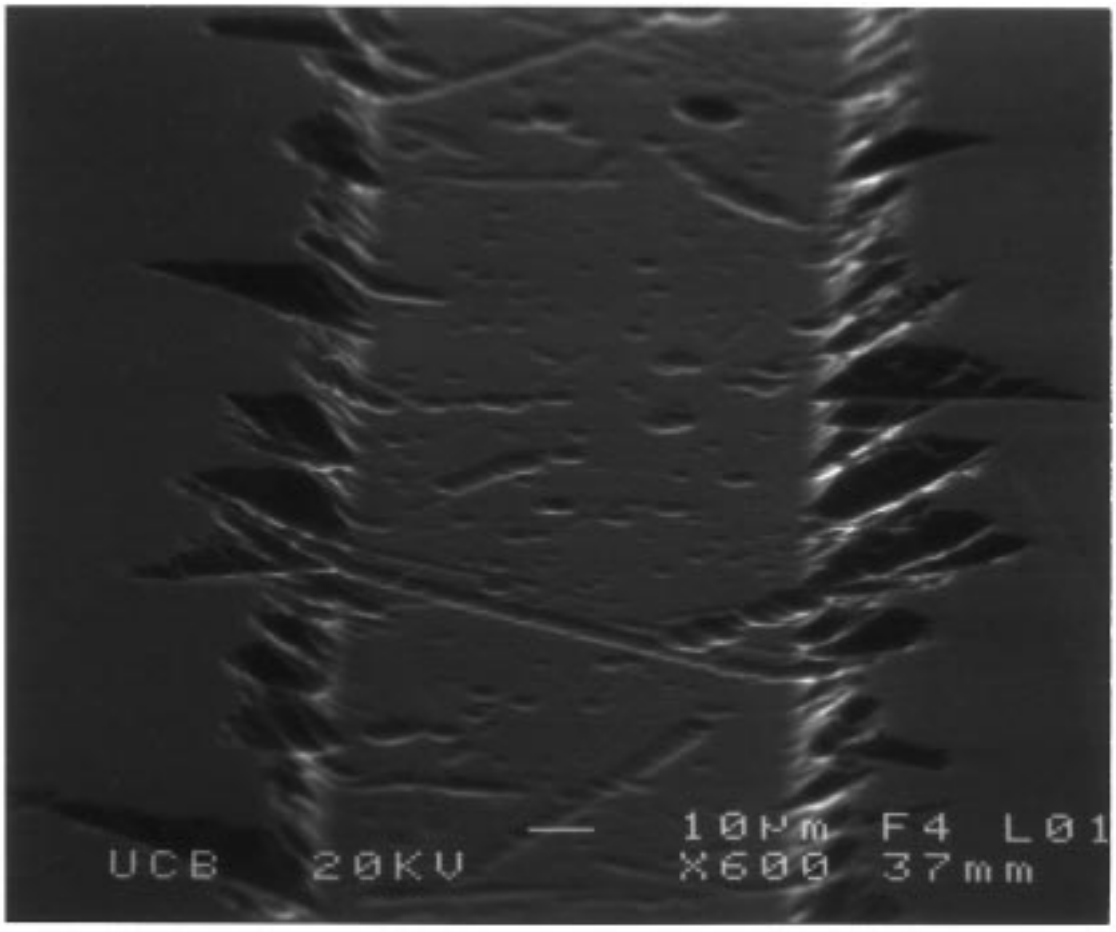
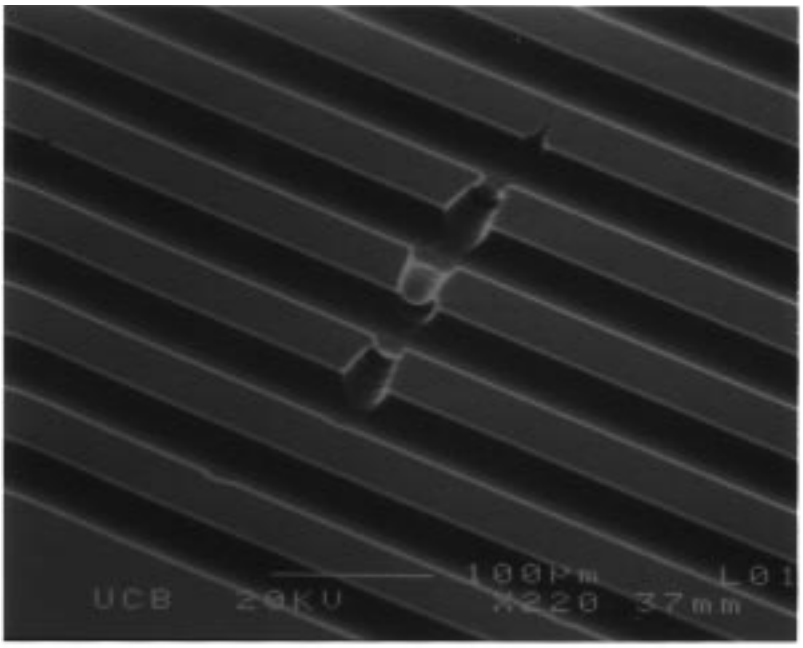


Fig. 4. *Scanning electron micrograph of polished Borofloat etched 7 μm using a Au/Cr etch mask. The patterns in the etched channel and defects in the side wall are due to polishing induced stresses and microscratches on the surface of the glass.*

polishing. The background fluorescence of this glass is also several times lower than that of microscope slides making it advantageous for high sensitivity experiments. The etch quality of Borofloat glass is shown in the electron micrograph in Figure 6. The glass etches at 7 μm/min. in 49% HF, does not require agitation during etching and leaves no reaction products as is seen with other types of glass such as soda lime. Moreover, because of its etch quality, bulk etching of the unprotected backside does not create distortions in the optical quality of the glass. Other glasses that we examined as possible substrates which yielded poorer results include Corning 1737, Corning 7059, Schott D263 and Schott AF45.

Glass drilling. The reservoirs that connect to the electrophoresis channels are drilled with diamond-tipped drill bits (Crystallite, Westerville, OH) using a hand drill press (Cameron Micro Drill Presses, Series 164, Sonora, CA). The pattern on the etched substrate is used as the template for the drilled holes. Drilling the holes through the etched substrate eliminates any problems of aligning separate etched and drilled wafers. Using a diamond-tipped drill bit to bore the access holes is much faster than ultrasonic drilling which can take as long as 15 minutes per hole; diamond tipped drilling takes only 15 seconds per hole. By drilling holes and using the etched pattern for alignment, devices with over 100 reservoirs have been fabricated. For production of many chips with a fixed hole pattern, ultrasonic drilling is advantageous because all the holes can be drilled simultaneously with a multi-tipped ultrasonic drill bit.

Glass bonding. The etched and drilled substrate is bonded to a blank substrate of the same size and type in an evacuated oven (J. M. NEY Company, Bloomfield, CT). The bonding is accomplished by placing the glass



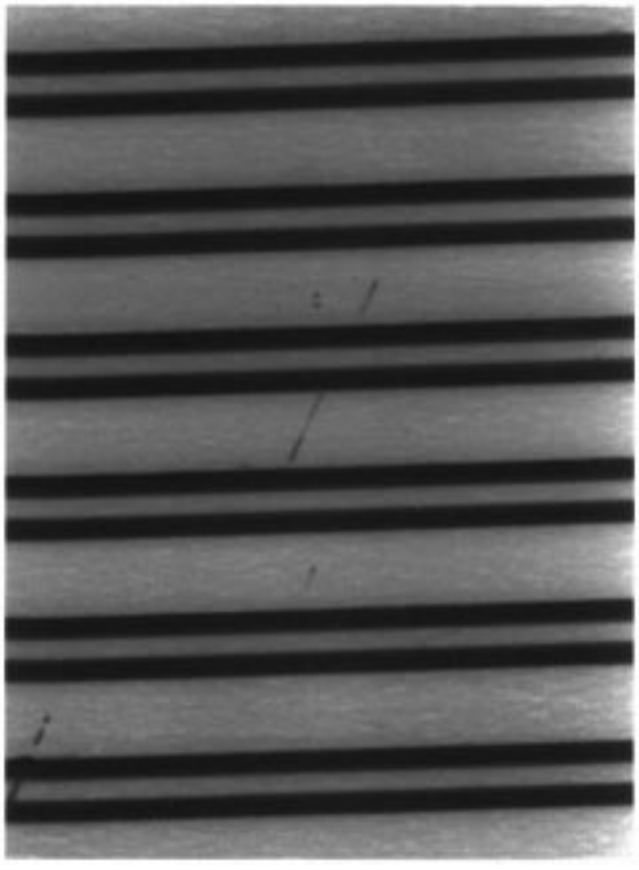


Fig. 5. *(A) Scanning electron micrograph showing the effect of a single scratch in the glass that occurred before deposition of the protective mask. The image shows an array of channels etched 20 µm deep in Borofloat using an amorphous silicon mask. (B) Optical image of the effect of a scratch defect in Borofloat where a pre-etch in 49% HF was performed prior to deposition of amorphous silicon and channel etching.*

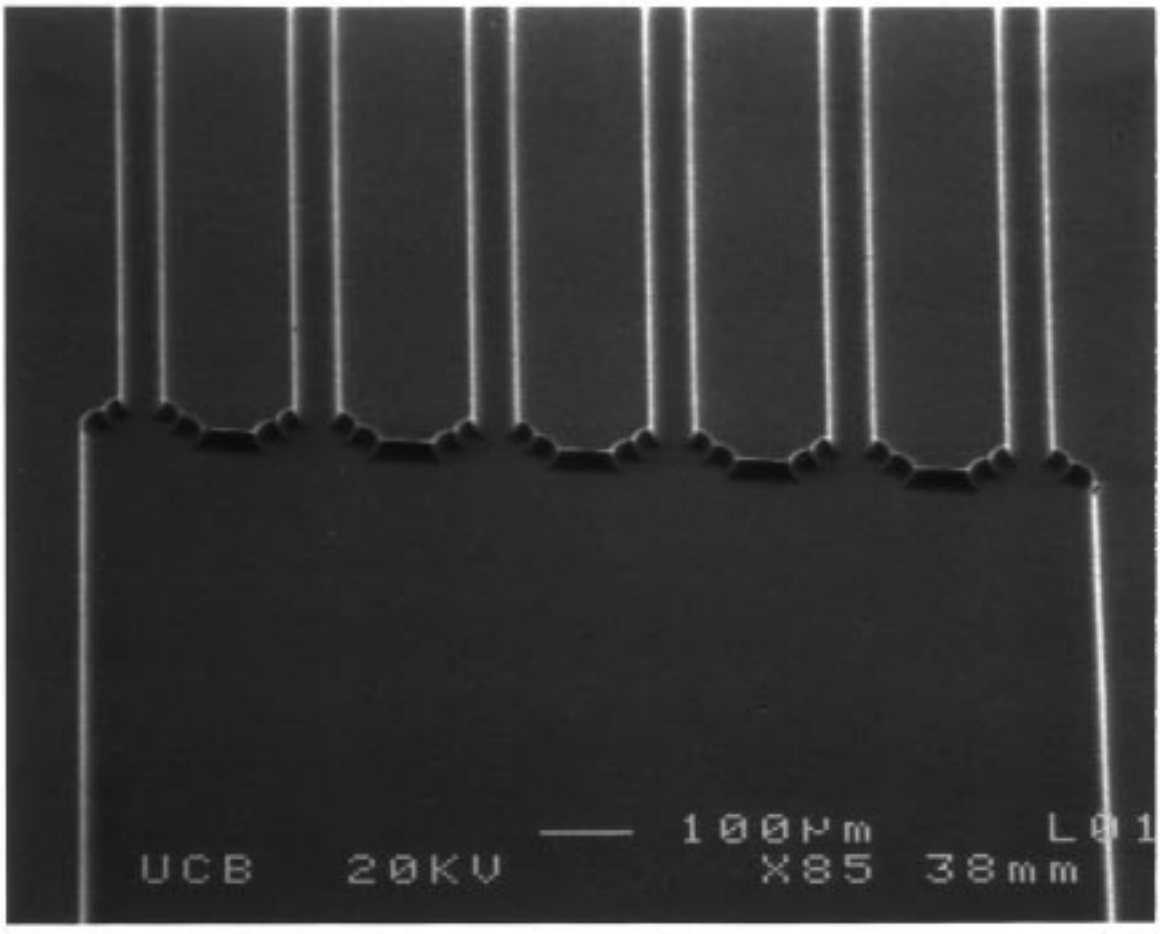


Fig. 6. *Scanning electron micrograph showing the quality of etches in Schott Borofloat glass. The image is of the common cathode region of a 48 sample chip etched 25 μm deep in 49% HF using amorphous silicon as an etch mask.*

substrates between two polished graphite blocks and placing a stainless steel weight (0.5 kg) on top. The furnace is then brought to above the annealing temperature of the glass and left at that temperature for several hours. For Borofloat, the temperature profile is: ramp up at 10°C/min. to 620°C, dwell time of 3.5 hours and ramp down at 10°C per minute. With well cleaned substrates and flat, polished graphite blocks, complete bonding over an entire 10 cm diameter substrate is achieved. An advantage of this method of bonding is that if a complete bond is not achieved, rebonding is possible with no deterioration of the substrate. Although care is taken to ensure clean surfaces, even the presence of a small amount of dust will affect the bonding quality only if it is in close proximity to an etched region. These improvements in chip fabrication are critical to the success of the more complex designs presented below.

High Throughput Parallel DNA Analysis Chips

Until our recent work [17], DNA separations in microfabricated chips were performed only on single channels [1,2,7,8]. However, for most applications, such as population screening or DNA sequencing, it is desirable to perform many separations in parallel. To begin exploring the issues involved in running multiple samples, we fabricated a chip with 12 parallel channels [17]. Figure 7 presents the layout of the twelve channel chip. Each separation channel possesses its own sample injection, cathode and sample waste reservoirs. The separation channels have a common anode reservoir, which reduces the total number of holes to $3N + 1$ required for a device with N separation channels.

The laser-excited confocal fluorescence scanner used for the CAE chip detection is shown in Figure 8 [22].

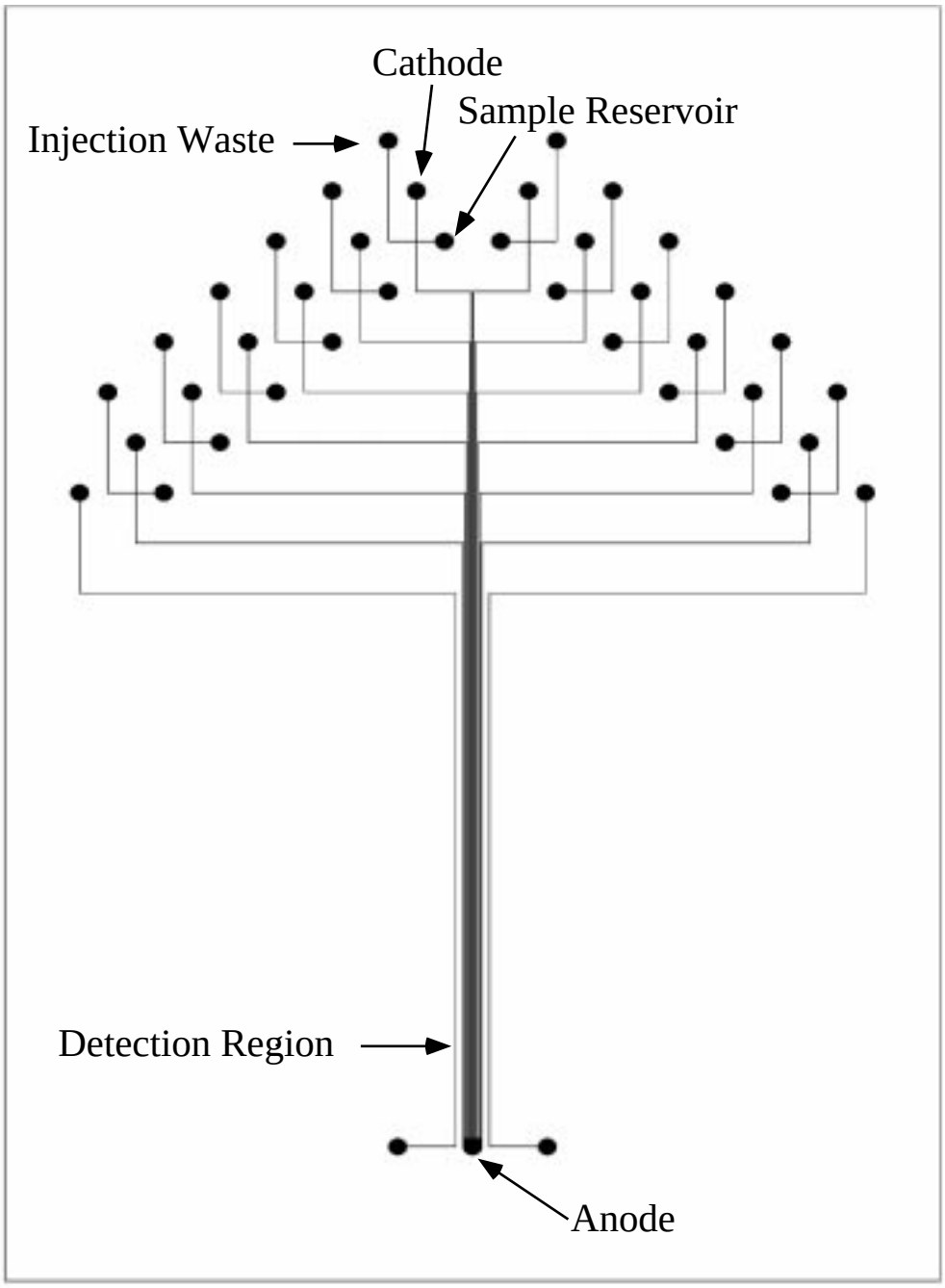


Fig. 7. *Mask design used to pattern the 12 channel CAE chip. Two optical alignment channels run parallel to the separation channels. The size of the pattern (region enclosed by the box) is 50 × 75 mm (Adapted from [17]).*

Briefly, the CAE chip was placed on a computer controlled translation stage, where the 488 nm line from an argon ion laser (∼ 5 mW) was focused within the separation channels. The individual separation channels were probed by translating the chip 1.2 mm back and forth through the focused beam at a rate of 3.3 Hz (1.0 cm/s). Fluorescence was collected by the objective, passed through a dichroic filter, and divided into two fluorescence channels with a second dichroic filter. The signal from both channels was focused with a lens onto a confocal spatial filter prior to photomultiplier detection.

Figure 9 presents electropherograms of 12 pBR322 *Msp*I restriction digests separated in parallel on a microfabricated CAE chip. The separations are

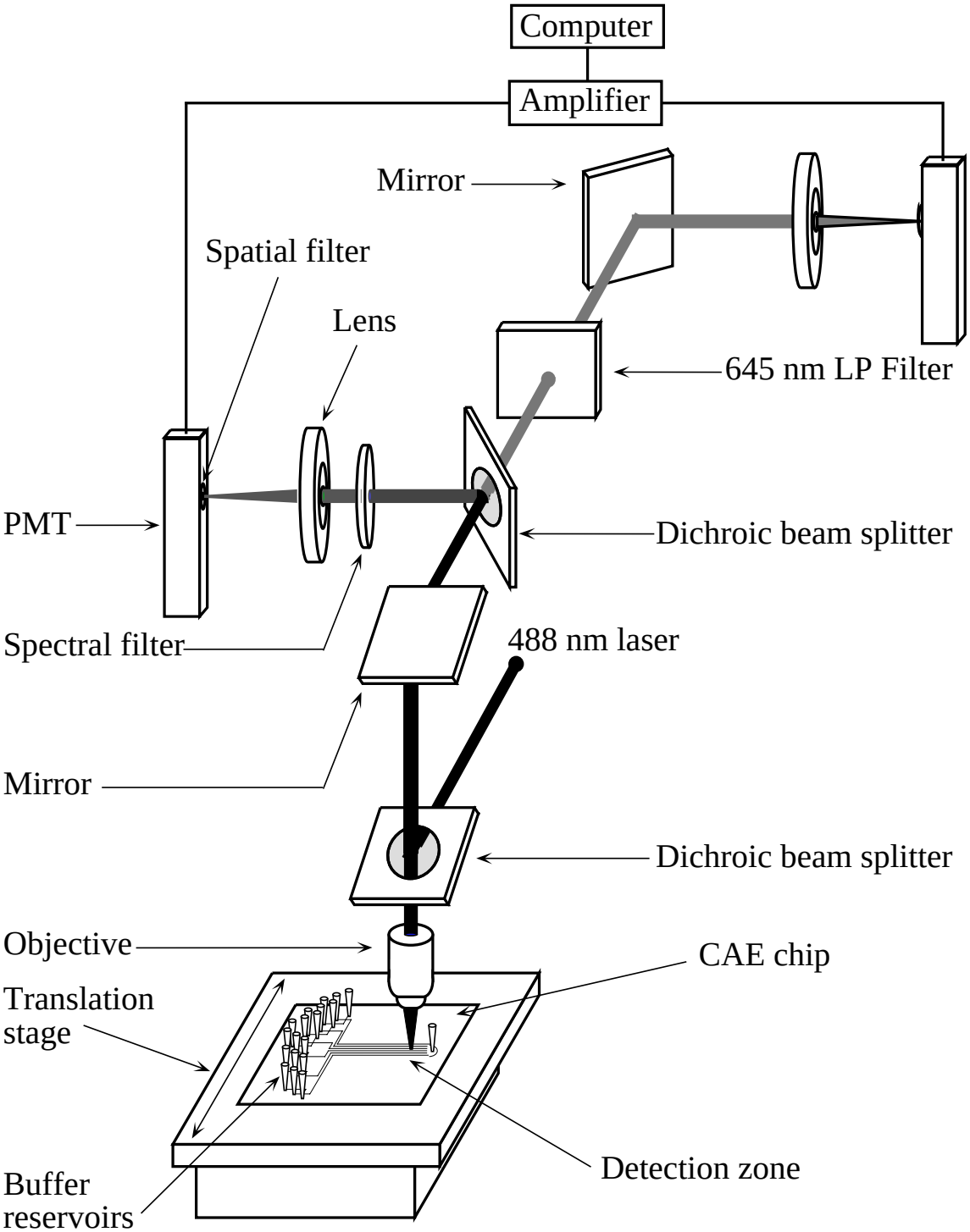


Fig. 8. *Schematic of the two-color, laser-excited confocal-fluorescence scanner used to detect the CAE chip (Reproduced with permission from [17]. Copyright 1997 A.C.S.).*

completed in < 160 s, or just 13 sec/sample. Consistent migration times from lane-to-lane (1.5% RSD, 12 measurements for the 622 bp fragments) were also obtained. These chips have also been used for genotyping of HLA-H amplicons, a candidate gene for the diagnosis of hereditary hemochromatosis [17], establishing the

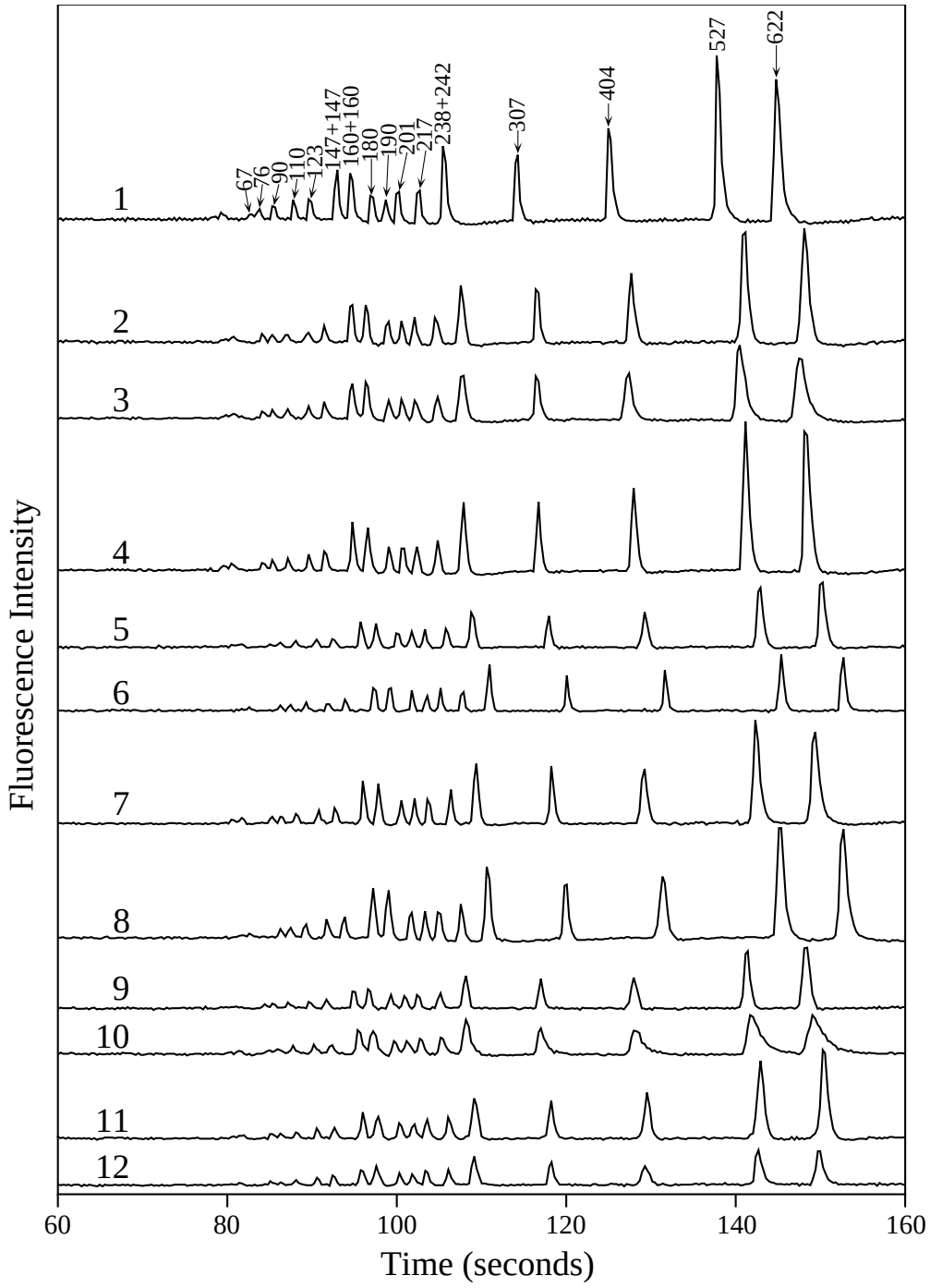


Fig. 9. *Electropherograms of pBR322 MspI DNA (2 ng/ul) separations performed in parallel on the 12 channel chip. The sizes of the DNA fragments are labeled next to the respective bands detected in lane 1. The average signal for each lane as a function of time is shown; the fluorescence intensity scale is the same for all 12 electropherograms (Reproduced with permission from [17]. Copyright 1997 A.C.S.).*

feasibility of CAE chips for rapid high throughput genetic analysis.

High throughput systems are needed for many applications where genetic screening of large populations is desired. If the twelve channel design could be expanded to analyze 96 samples, a single microfabricated 96 sample CAE chip would have a higher sample capacity than any commercial automated slab gel

apparatus and would be orders of magnitude faster. One of the challenges for running a 96 sample CAE chip is the method of detection. The 12 channel chip was scanned bidirectionally with periodic 0.3 sec. sweeps at a scan rate of 1.0 cm/sec. This gave a sampling rate of 3.3 Hz for a scanned region width of 1.2 mm. However, if the scanned region is increased by adding 84 additional channels, this scanning method will no longer be practical due to the larger mechanical motion required and the reduced sampling rate per channel. Other methods of detection such as galvanometric scanning [23] or CCD systems [24,25] may offer improved temporal resolution, but the sensitivity of such detection systems has not been demonstrated.

Multiple injections

Since separation times are fast on chips (<160 sec.) an alternative approach for increasing throughput is to separate multiple samples serially on the same capillary. We have explored the injection of two, four and eight samples on a single separation capillary. Preliminary work using two injection reservoirs per channel is presented in Figure 10. The layout of the two reservoir channel capable of injecting two samples is presented in Figure 10A. First, a 268 bp β-globin PCR amplicon was injected by applying a potential between sample reservoir 1 and the injection waste reservoir while allowing sample 2 to float. Then a ΦX174 *Hae*III ladder was injected by applying the potential between sample 2 and the waste reservoirs and allowing sample 1 to float. Finally, both samples were injected at the same time by applying the field to both the sample 1 and sample 2 reservoirs. The electrophoretic co-injection of a PCR target and a standard ladder is useful for size calibration. This work demonstrates the feasibility of serial analysis of multiple DNA samples by a single capillary on a microfabricated CE chip.

In order to maximize sample throughput while keeping the separation time low, four injection reservoirs per channel was chosen for the design of high throughput array chips. Figure 11A shows the layout of a single capillary capable of injecting 4 different samples. Injection waste B is used when injecting from sample reservoirs 1 and 2 while injection waste A is used to inject from sample reservoirs 3 and 4. In order to avoid contamination, direct voltage control of four reservoirs (anode, cathode, sample injection and injection waste) is necessary during injection and electrophoresis. The other three sample reservoirs and the additional injection waste reservoir are allowed to float. Four high voltage power supplies (Stanford Research Systems, Series PS300, Sunnyvale, CA) controlled by a computer program created in LabVIEW (National Instruments, Austin, TX) are used to apply the potentials to the reservoirs and to time the injection. Figure 11B shows the electrokinetic injection of 100 μM fluorescein from sample reservoir 1. Since the walls of the channels have been coated with linear polyacrylamide [26], no electroosmotic flow occurs. Fluorescein and DNA are both negatively charged at pH 8.3 so fluorescein is an effective method of visualizing the movement of DNA in applied fields. During injection, 300 V/cm is applied between injection reservoir 1 and injection waste B. Anions, including fluorescein and DNA, migrate from reservoir 1 towards B. The anode and cathode reservoirs are kept at a voltage $0.7\times$ the calculated voltage (approximated from the 1 and B voltages) of the intersection region. By applying fields at the anode and cathode, the electric field in the injection region controls the width of the injection plug. Using this method the injection width is steady over time, allowing for injections up to 90 seconds which ensures complete injection of all analytes independent of mobility. Although this injection method is effective in controlling the width of the injection plug, the signal from the injected material is decreased by as much as an order of magnitude relative to unbiased injection.

When the electric fields are switched from the injection to run mode, 150 V/cm are applied between anode and cathode reservoirs and a back biasing potential of $1.5\times$ the calculated potential (approximated from anode and cathode voltages) at the intersection region are applied at reservoirs 1 and B. Figure 11C shows the injection region in run mode where a plug of fluorescein is traveling down the electrophoresis channel while the excess analyte is pulled back towards the sample 1 and injection waste B reservoirs. The back biasing prevents bleeding of sample from the injection channel into the electrophoresis channel and removes sample 1 from the injection channel to prevent contamination of subsequent injections.

One of the major issues associated with injecting multiple samples onto the same capillary is the potential for run-to-run contamination. The potentials of reservoirs 1, injection waste B, anode and cathode are controlled during the first injection while the remaining reservoirs are allowed to float. Diffusion of the sample into the other channels occurs during the first injection creating potential cross contamination sites. When the next sample is injected, the contamination is pulled through the injection region and on towards the injection waste reservoir before the second sample approaches the injection cross channel. In order to look for the presence of cross contamination, Figure 12 shows 10 nM fluorescein injected from reservoir 1, a buffer blank injected from reservoir 2, 1 nM fluorescein injected from reservoir 3 and again a buffer blank injected from reservoir 4. No peaks were observed for injections 2 and

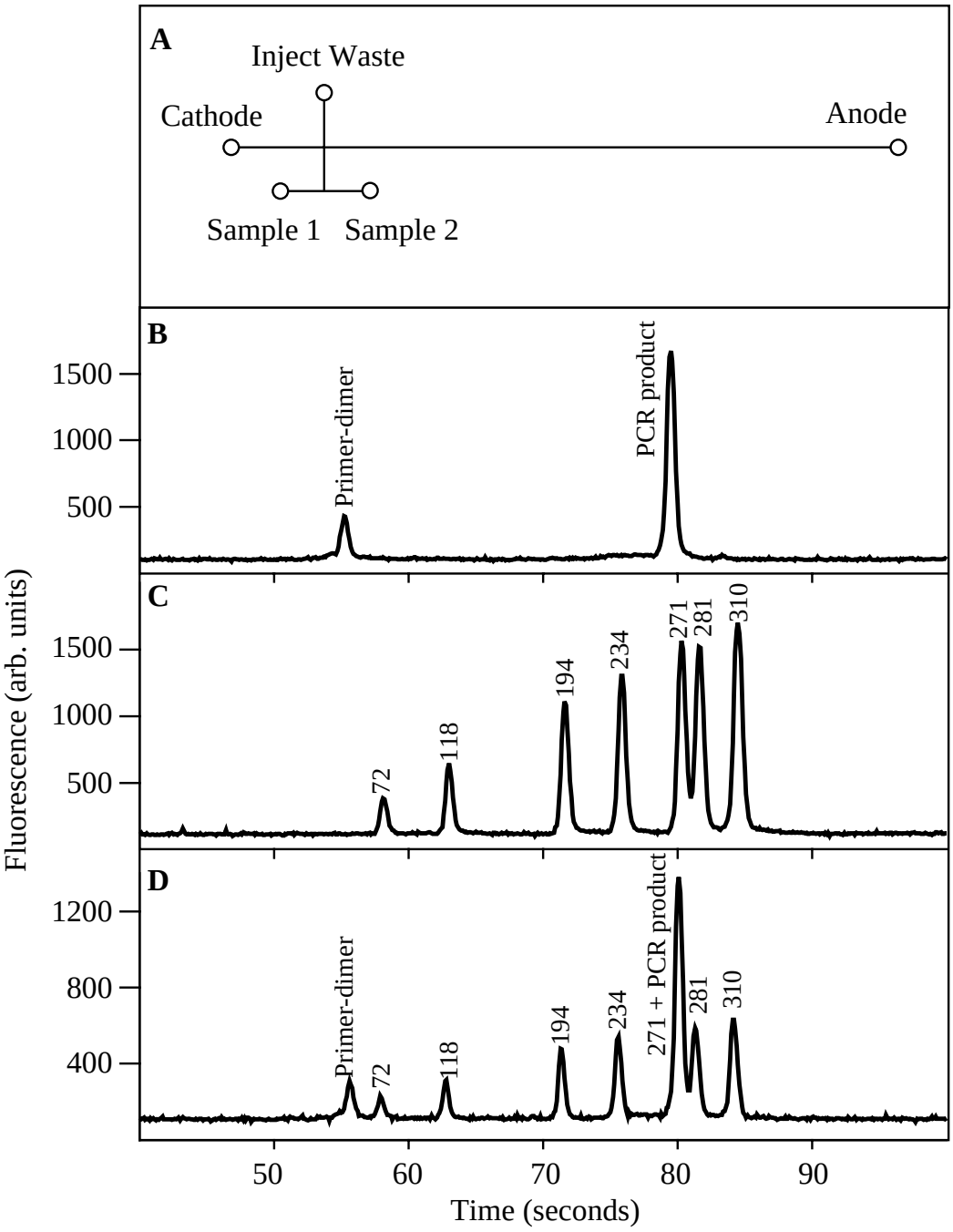


Fig. 10. *(A) Layout of a two injection reservoir channel. Electropherograms showing serial separations of (B) a 268 bp β-globin PCR product, (C) the smaller peaks of ΦX174 HaeIII ladder and (D) the separation of both samples co-injected.*

4 (lower limit 35 pM for S/N of 3), confirming the absence of cross contamination. Finally, because of the relatively long distance (3 mm) between the reservoirs and channel intersections, the loaded chip can be left for considerable time (> 90 min.) without any evidence of diffusional contamination.

Using a four sample injection chip, four DNA digests were analyzed in series. Figure 13 shows the separation of ΦX174 *Hae*III, pBR322 *Bst*N I, pBR322 *Msp*I and ΦX174 *Hae*III injected from sample reservoirs 1, 2, 3 and 4 respectively; the separations were performed in approximately 10 minutes.

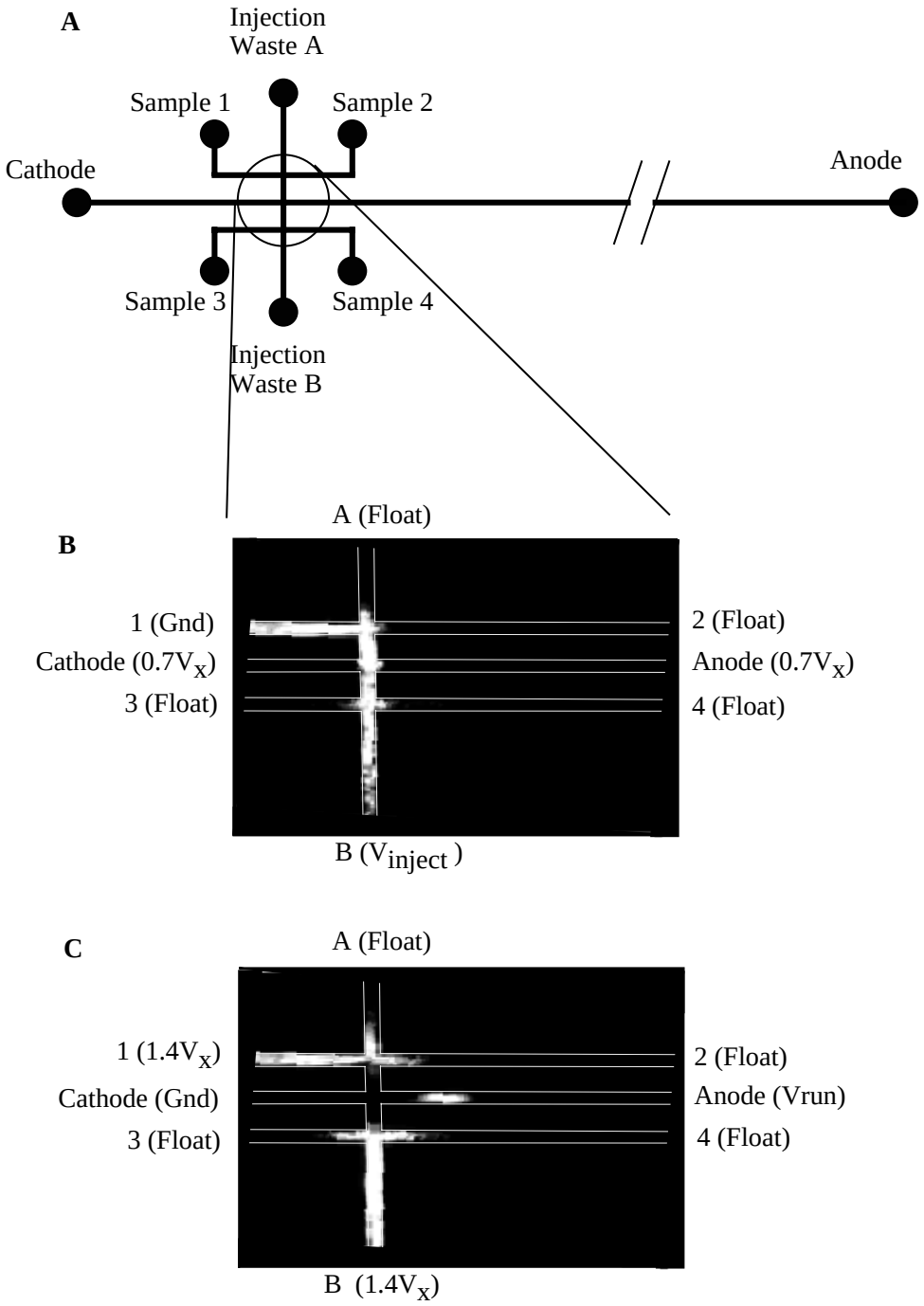


Fig. 11. *(A) Schematic of a multi-sample injection channel where four samples can be injected and separated on a single capillary. (B) Fluorescence image of the sample flow of fluorescein during inject mode. V_x is the calculated voltage at the cross channel. (C) Fluorescence image of the sample flow during the run mode. An injection plug of fluorescein is electrophoresed down the channel to the right while back biasing potentials pull the excess sample back into reservoir 1 and injection waste B.*

48 Sample chips

The serial injection of multiple samples onto multiple capillaries allows for high throughput systems while still permitting adequate sampling from the detection system. Figure 14 shows a 48 sample chip design that is based on injection of 4 samples serially onto 12 capillaries. The capillaries feed into one of 2 common cathode reservoirs and share a common anode reservoir, allowing analysis

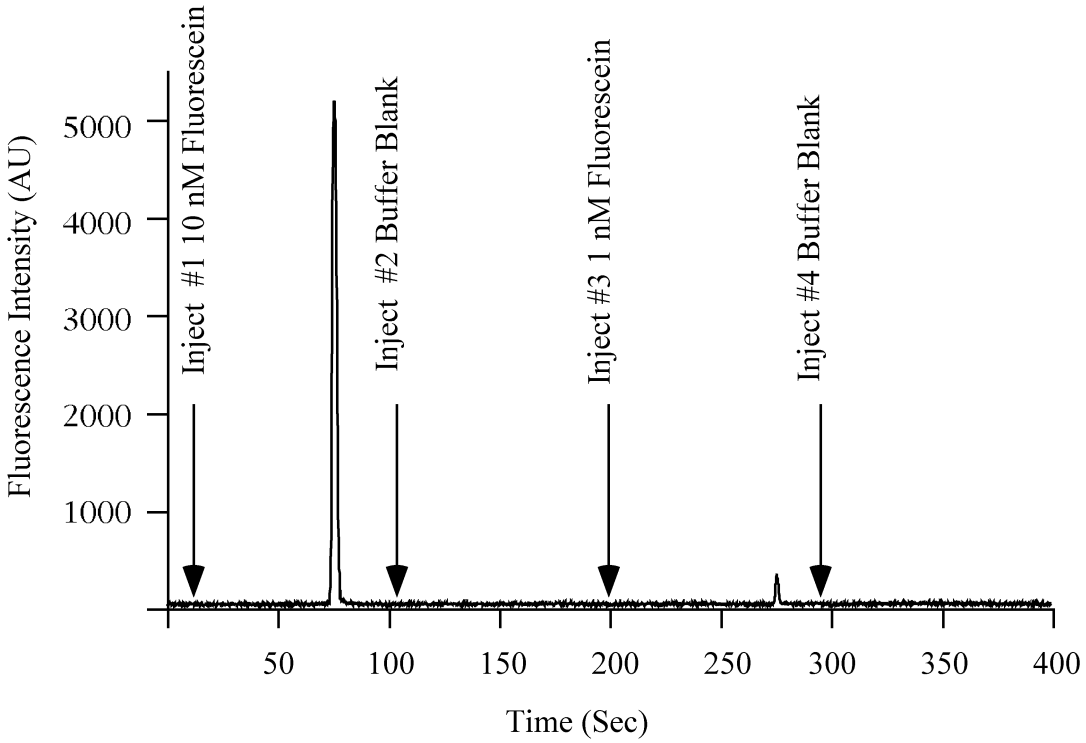


Fig. 12. *Electropherogram studying the effects of contamination in the four sample capillary. The figure shows the separation of 10 nM fluorescein injected from reservoir 1, a buffer blank injected from reservoir 2, 1 nM fluorescein injected from reservoir 3 and again a buffer blank was injected from reservoir 4.*

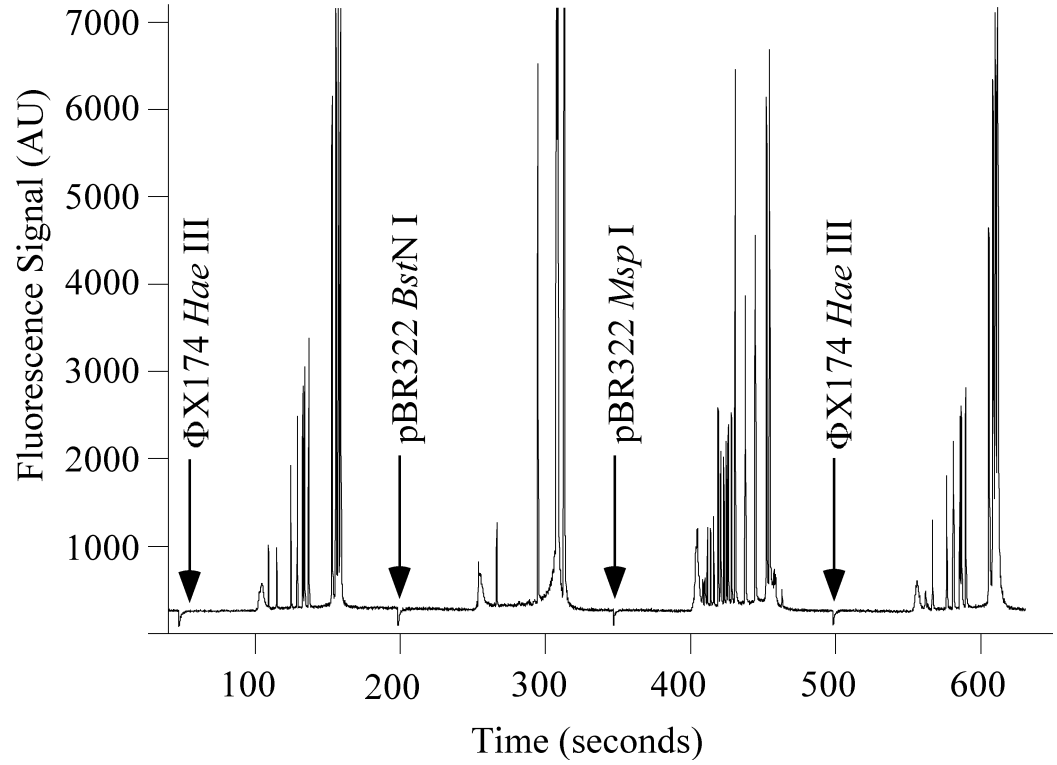


Fig. 13. *Serial injection and separation of four DNA digests on a single CE chip channel; ΦX174 Hae III, pBR322 BstN I, pBR322 MspI and ΦX174 HaeIII in order of analysis.*

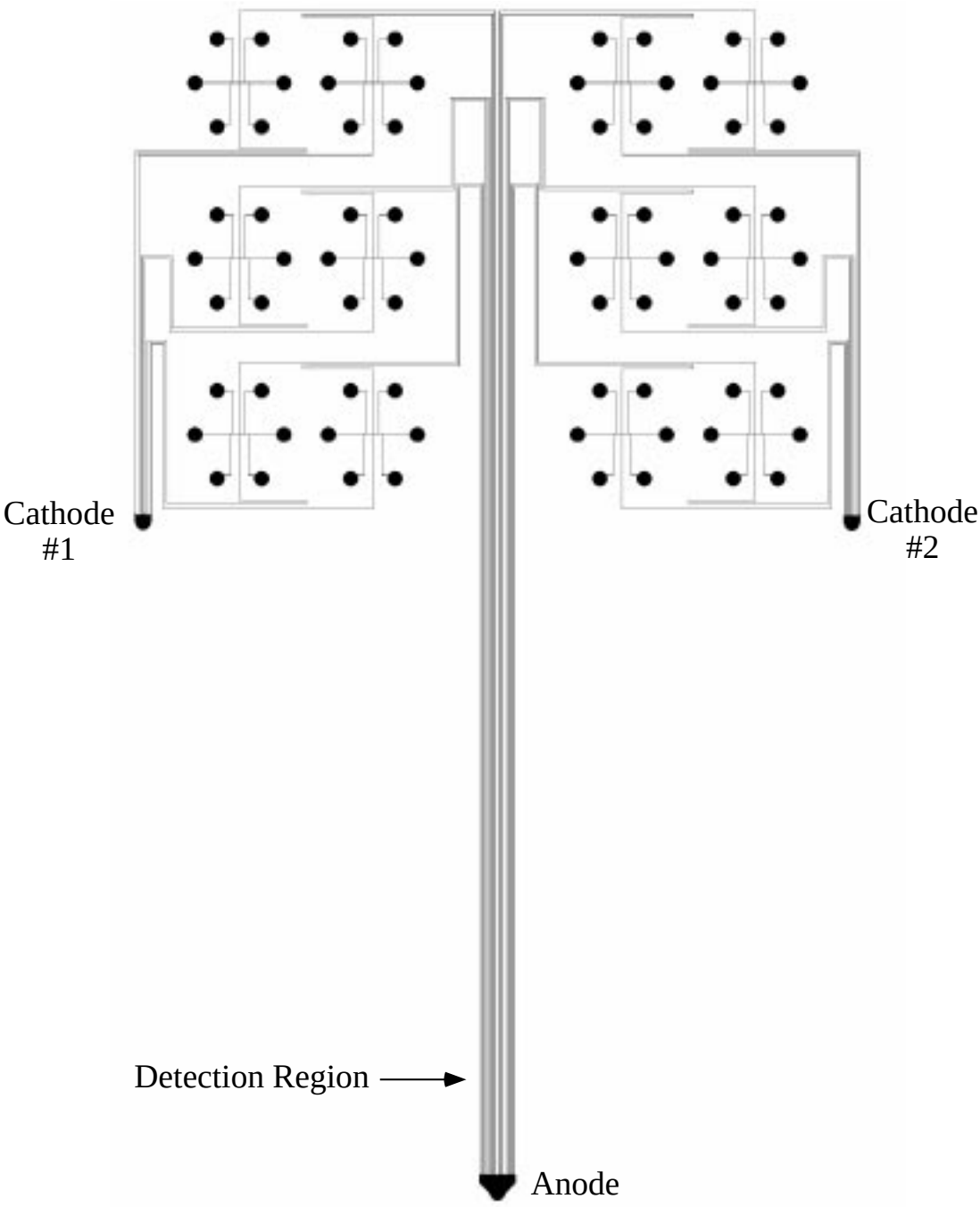


Fig. 14. *Mask design for a 48 sample chip. The chip has 12 separation channels which allow injection of four samples per channel. Two common cathodes and one anode are used to decrease the total number of holes. The pattern fits onto a 10 cm glass wafer.*

of 48 samples with just 75 reservoirs. The distances from the anode and to the injection region and from the cathode to the injection region are the same for all channels. The injection reservoirs are placed equidistant in the vertical dimension to allow automated parallel loading.

In order to actually run a 48 sample chip, simplified sample loading methods are required. In the 12 channel chip, cut off pipette tips were used as reservoirs to hold the samples and buffers. This approach is impractical with 48 samples, as evaporation of the numerous samples would be a major issue. Additionally, placing pipette tips

and individual wires in > 100 reservoirs would be tedious. To simplify the procedure, a sample loading assembly was fabricated, consisting of an array of electrodes set on top of a loading array of sample reservoirs (Figure 15). The loading array consists of 1.5 mm holes punched in a cast sheet of 1/8 inch thick PDMS elastomer (Corning, Sylgard 184) [27]. Using positive pressure, the chip was filled first with 0.8% HEC from the common anode, and then, the two common cathodes to ensure complete filling. After completely filling the reservoirs, the HEC in the sample reservoirs was removed and replaced with the sample. The electrode array was then placed on top to establish electrical contact and to reduce evaporation. In this manner, 48 samples of fluorescein were injected and analyzed in less than 20 minutes. The increased time required for this separation, relative to previous CE chip work, is due to lower applied fields and the longer electrophoresis length. Figure 16 demonstrates the separation of 48 samples consisting of four different concentrations of fluorescein, corresponding to each of the four different injection reservoirs.

Conclusions

To fabricate and run higher throughput capillary array electrophoresis chips, improvements in both the fabrication and sample handling were necessary. By exploring improved, more resistant etch masks and by using Borofloat glass, we are now able to routinely fabricate arrays of capillaries on 10 cm diameter substrates. High throughput CAE chips have been successfully fabricated and used to analyze up to 48 samples on a single device with scanned confocal detection. Sample loading and electrical addressing issues are solved by using elastomer structures to form the sample wells and to introduce the electrodes. With these improvements, CAE chips will become practical tools for high speed, high throughput genetic analysis. Our future work will explore the separation of biologically relevant samples on high throughput chips and the extension of this CAE chip methodology to 96 samples.

Acknowledgments

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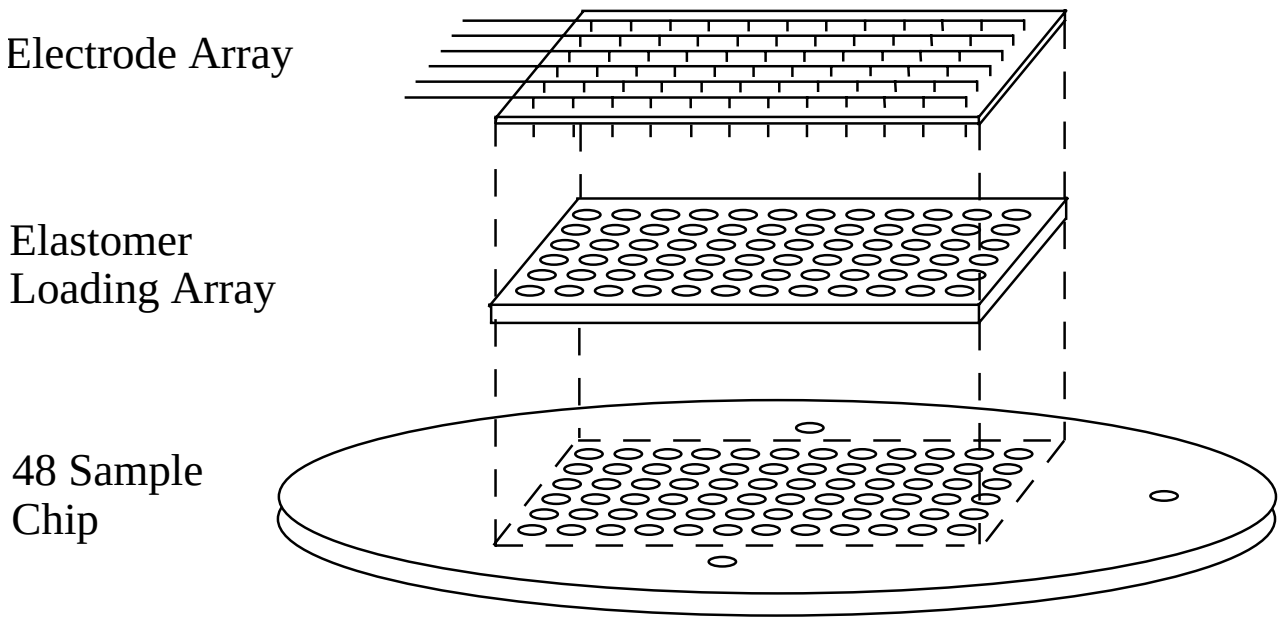

Fig. 15. *Schematic of the elastomer loading array and electrode array.*

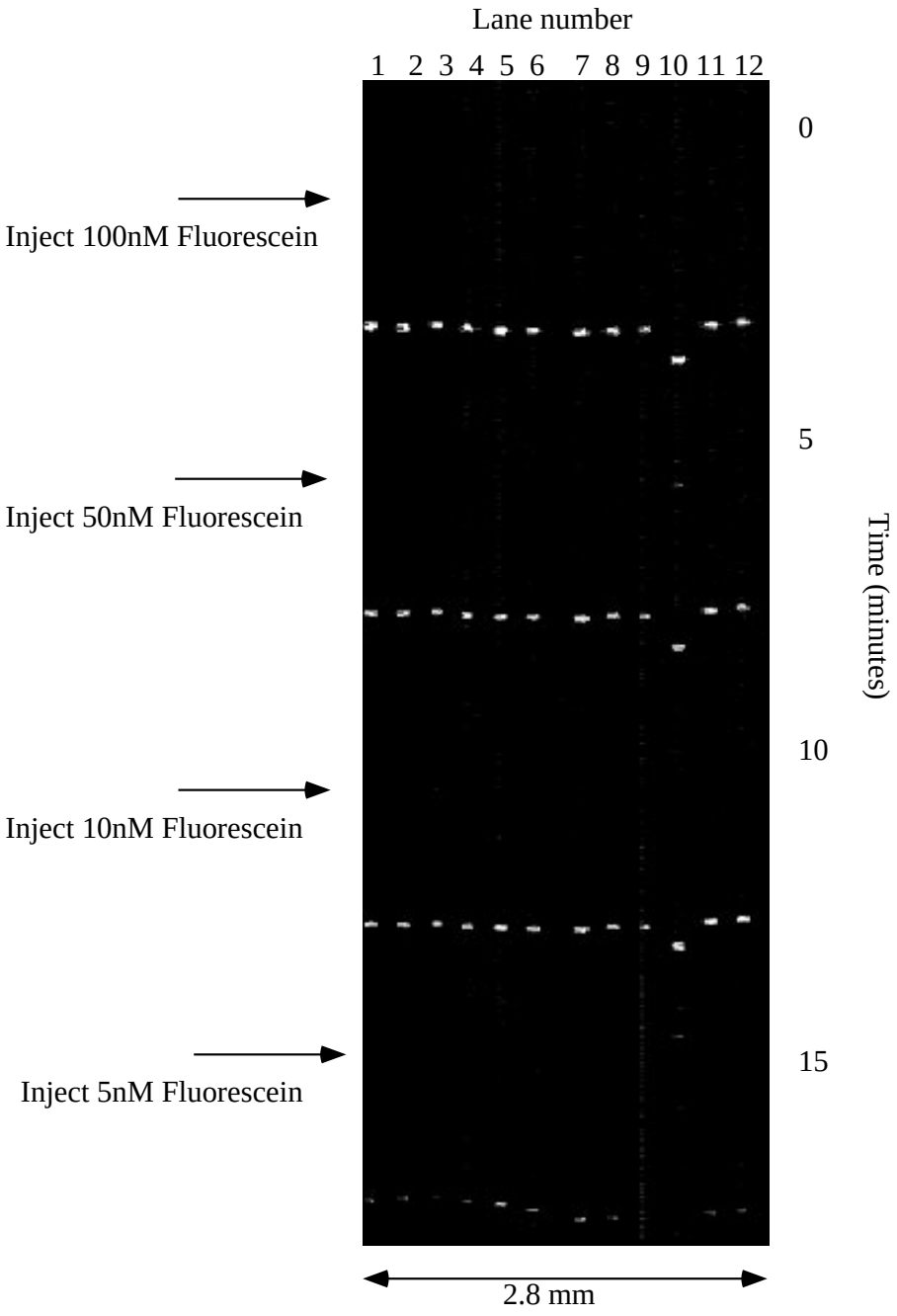

Fig. 16. *Image of the separation of 48 samples of fluorescein on a microfabricated CAE chip. Four concentrations of fluorescein were separated in series on 12 channels. The fluorescein concentrations are 100, 50, 10 and 5 nM respectively. Total separation time is about 20 minutes.*

References

1. D.J. Harrison, A. Manz, Z. Fan, H. Ludi, and H.M. Widmer, Anal. Chem. **64**, 1926–1932 (1992).
2. S.C. Jacobson, R. Hergenroeder, L.B. Koutny, R.J. Warmack, and J.M. Ramsey, Anal. Chem. **66**, 1107–1113 (1994).
3. D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, and A. Manz, Science **261**, 895–897 (1993).
4. C.S. Effenhauser, A. Manz, H.M. Widmer, Anal. Chem. **65**, 2637–2642 (1993).
5. S.C. Jacobson, R. Hergenroder, A.W. Moore., and J.M. Ramsey, Anal. Chem. **66**, 4127–4132 (1994).
6. S.C. Jacobson, A.W. Moore, and J.M. Ramsey, Anal. Chem. **67**, 2059–2063 (1995).
7. L.B. Koutny, D. Schmalzing, T.A. Taylor, and M. Fuchs, Anal. Chem. **68**, 18–22 (1996).

8. A.T. Woolley and R.A. Mathies, Proc. Natl. Acad. Sci. **91**, 11348–11352 (1994).
9. S.C.Jacobson and J.M. Ramsey, Anal. Chem. **68**, 720–723 (1996).
10. C.S. Effenhauser, A. Paulus, A. Manz, and H.M. Widmer, Anal. Chem. **66**, 2949–2953 (1994).
11. A.T. Woolley and R.A. Mathies, Anal. Chem. **67**, 3676–3680 (1995).
12. M.A. Northrup, M.T. Ching, R.M. White, and R.T. Watson, *Digest of Technical Papers: Transducers 1993* (IEEE, New York, NY, 1993), pp. 924–926.
13. M.A. Burns, C.H. Mastrangelo, T.S. Sammarco, F.P. Man, J.R. Webster, B.N. Johnson, B. Foerster, D. Jones, Y. Fields, A.R. Kaiser, and D.T. Burke, Proc. Natl. Acad. Sci. U.S.A. **93**, 5556–5561 (1996).
14. A.T. Woolley, D. Hadley, P. Landre, A.J. deMello, R.A. Mathies, and M.A. Northrup, Anal. Chem. **68**, 4081–4086 (1996).
15. Z. Liang, N. Chiem, G. Ocvirk, T. Tang, K. Fluri, and D.J. Harrison, Anal. Chem. **68**, 1040–1046 (1996).
16. R.A. Mathies and X.C. Huang, Nature **359**, 167–169 (1992).
17. A.T. Woolley, G.F. Sensabaugh, and R.A. Mathies, Anal. Chem. **69**, 2181–2186 (1997).
18. K. Fluri, G. Fitzpatrick, N. Chiem, and D.J. Harrison, Anal. Chem. **68**, 4285–4290 (1996).
19. Z.H. Fan and D.J. Harrison, Anal. Chem. **66**, 177–184 (1994).
20. P.C. Simpson, *Capillary Electrophoresis Chip Fabrication and Improvements in DNA Sequencing Resolution*, M. Eng. Degree Project, Cornell University, 1996.
21. G.R. Cokelet, R. Soave, G. Pugh, and L. Rathbun, Microvascular Research **46**, 394–400 (1993).
22. I. Kheterpal, J.R. Scherer, S.M. Clark, A. Radhakrishnan, J. Ju, C.L. Ginther, G.F. Sensabaugh, and R.A. Mathies, Electrophoresis **17**, 1852–1859 (1996).
23. O. Trepte and A. Liljeborg, Opt. Eng. **33**, 3774–3780 (1994).
24. We evaluated CCD detection of the CAE chips using a line focused laser beam. We found that rather high laser power ($\sim$ 300 mW) was necessary to optimally excite fluorescence in the channels and that background fluorescence from the CAE chip reduced the signal-to-noise ratio. The DNA detection sensitivity with the CCD was therefore about $10\times$ worse than with the confocal detection system, primarily because of the higher background glass fluorescence due to poorer spatial filtering with the CCD system. The scanning confocal system was therefore preferred because of its better detection limits and lower laser power requirements.
25. R.L. Brumley, Jr. and L.M. Smith, Nucleic Acids Res. **19**, 4121–4126 (1991).
26. S. Hjerten, J. Chromatogr. **347**, 191–198 (1985).
27. Y. Xia, E. Kim, X.-M. Zhao, J.A. Rogers, M. Prentiss, and G.M. Whitesides, Science **273**, 347–349 (1996).