



Published in final edited form as:

Anal Chem. 2008 September 15; 80(18): 7153–7157. doi:10.1021/ac801059h.

Calcium-Assisted Glass-to-Glass Bonding for Fabrication of Glass Microfluidic Devices

Peter B. Allen and Daniel T. Chiu*

Department of Chemistry, University of Washington, Box 351700, Seattle, Washington 98195

Abstract

Glass is a desired material for many microfluidics applications. It is chemically resistant and has desirable characteristics for capillary electrophoresis. The process to make a glass chip, however, is lengthy and inconvenient, with the most difficult step often being the bonding of two planar glass substrates. Here we describe a new glass bonding technique, which requires only washing of the glass surfaces with a calcium solution and 1–2 h of bonding at 115 °C. We found calcium uniquely allows for this simple and efficient low-temperature bonding to occur, and none of the other cations we tried (e.g., Na⁺, Mg²⁺, Mn³⁺) resulted in satisfactory bonding. We determined this bond is able to withstand high applied field strengths of at least up to 4 kV · cm⁻¹. When intense pressure was applied to a fluid inlet, a circular portion of the coverslip beneath the well exploded outward but very little of the glass–glass interface debonded. In combination with the directed hydrofluoric acid etching of a glass substrate using a poly(dimethylsiloxane) (PDMS) etch guide, we were able to make glass chips with better than 90% yield within 6 h. This technique is compatible with inexpensive unpolished glass and is limited in resolution by the PDMS etch guide used and the intrinsic properties of isotropic etching.

The best established method for making glass chips is to etch a substrate with hydrofluoric acid (HF) or reactive ion etch and to bond a cover of the same material via thermal fusion.¹ Anodic bonding is a popular method for silicon–glass devices but requires the deposition of a layer of silicon to one glass surface for glass–glass bonding.² Other kinds of adhesive layers have been demonstrated but can undermine the advantages of the glass material.³ Extremely clean and flat glass will adhere by van der Waals interactions when brought into contact,⁴ and after lengthy cleaning procedures, this has been utilized for low-temperature glass–glass sealing.^{5–8} These low-temperature sealing techniques permit the bonding of two pieces of glass that differ in their respective coefficient of thermal expansion (CTE).

This paper introduces a new low-temperature bonding technique, which relies on washing the glass surfaces with a calcium solution, to rapidly produce an all-glass chip with high reproducibility from inexpensive, unpolished glass. We have produced features in glass using a poly(dimethylsiloxane) (PDMS) etch guide for HF.⁹ One molded PDMS guide can be used for multiple chips rather than a new lithographic process or even a replication

*To whom correspondence should be addressed. chiu@chem.washington.edu.

SUPPORTING INFORMATION AVAILABLE

Detailed procedure with instructive images and diagrams. This material is available free of charge via the Internet at <http://pubs.acs.org>.

molding step for each new chip. Normally, small PDMS channels are too hydrophobic to wet with aqueous HF, but we implemented a method for altering the surface properties of PDMS to permit the generation of small glass features. We show that this approach has a resolution limit comparable to that for PDMS with consideration for the inherent limitations of any isotropic etching technique. We demonstrate the use of this technique by fabricating a microchip for capillary electrophoresis (CE) separation.¹⁰ For this chip, we etched a standard microscope slide to which we bonded a standard coverslip with an unmatched coefficient of thermal expansion.

Our need to apply high separation voltages to achieve fast CE separation motivated us to explore new, rapid prototyping techniques for inexpensive glass substrates. PDMS-based microfluidic systems, although easy to fabricate and well suited for a wide range of applications, cannot withstand field strengths greater than $\sim 600\text{--}800\text{ V}\cdot\text{cm}^{-1}$. To test the strength of our Ca^{2+} -assisted glass–glass bond, we show that our finished chip is stable up to $4\text{ kV}\cdot\text{cm}^{-1}$, and the measured electroosmotic flow (EOF) rate at this high field strength is very reproducible and stable. We did not test higher field strengths, because these higher voltages produced arcing between the electrodes. For the occasional times when arcing did not occur at higher voltages, we saw the glass chips withstood up to $10\text{ kV}\cdot\text{cm}^{-1}$. Nonetheless, $4\text{ kV}\cdot\text{cm}^{-1}$ exceeds the practical limitation for PDMS ($600\text{--}800\text{ V}\cdot\text{cm}^{-1}$) by a wide margin and is more than sufficient for most CE separations. Furthermore, under high pressure applied to a fluid inlet, we observed that a circular portion of the coverslip beneath the well would explode outward but very little of the glass–glass interface would debond.

In addition to low-temperature Ca^{2+} -assisted bonding, we also present the results of an expanded technique that incorporates high-temperature fusion bonding. Here we bonded a glass substrate and coverslip with a matched CTE, first with Ca^{2+} -assisted bonding, after which we placed the chip in a high-temperature furnace for fusion bonding. The advantage to doing this two-step process rather than direct fusion bonding is that we can inspect the chip during the intermediate, sealing stage and correct for any defects that might have existed during the initial glass–glass seal. Chips bonded by thermal fusion¹¹ have been shown to tolerate field strengths of $110\text{ kV}\cdot\text{cm}^{-1}$, so this expanded bonding step can be employed for applications that involve such extreme voltages.

MATERIALS AND METHODS

Fabrication of a PDMS Etch Guide

Using methods described extensively elsewhere,¹² we prepared a PDMS channel system with the mirror image of the desired pattern in glass. We included the grid-work pattern described by Stjernstrom and Roeraade,¹³ which helps prevent propagation of any bond defects. Briefly, photomasks with the desired channel designs (Figure 1A) were drawn up in Autocad (Autodesk, San Rafael, CA) and generated using a photoplotter (Photoplot Store, Colorado Springs, CO). We chose to implement this design as a two-layer fabrication in order to avoid low-aspect-ratio structures that might sag in PDMS. Prior to photolithography using the first photomask, SU-8-2050 photoresist (Microchem, Newton, MA) was spin-coated onto a silicon wafer at $30\text{-}\mu\text{m}$ thickness to form layer-one features on the silicon wafer (Figure 1A). Following exposure, curing, and development of this layer-one feature,

SU-8-2100 was spin-coated onto the same wafer at 100- μm thickness and exposed using a second photomask to define the features for layer two of the SU-8-on-silicon master. Following curing and development, the finished two-layer master was coated with (tridecafluoro-1,1,2,2-tetrahydrooctyl-) trichlorosilane (Gelest, Morrisville, PA) by gas-phase deposition to prevent sticking of PDMS during molding and peel-off. PDMS (Sylgard 184, Dow Corning, Midland, MI) was cast on this master and peeled away with recessed channels.

For features below 50 μm in width, we treated the PDMS chip with amino propyl triethoxy silane (APTES, Sigma-Aldrich, St. Louis, MO) by gas-phase deposition. The PDMS replica was exposed to oxygen plasma. We utilized a plasma cleaner (PDC-001, Harrick Plasma, Ithaca, NY) purged with oxygen twice and then pumped to 200 mTorr. We then oxidized on a “high” power setting (nominally, 29.6 W) for 2 min after initial plasma was observed. The oxidized PDMS was then transferred immediately to a desiccator with a vial containing ~50 μL of APTES. The desiccator was evacuated with a mechanical vacuum pump for 1–2 min. The PDMS was allowed to react with gaseous APTES for at least 2 h, preferably overnight. We spotted 10% HF solution on APTES-treated PDMS as well as on untreated PDMS. The contact angles were 61° and 91°, respectively.

Etching, Cleaning, and Sealing Glass Chips

The molded PDMS replica was punched with holes to define reservoirs for fluid inlets and outlets. We cut a PDMS “dam” from a featureless slab of PDMS. This dam comprised a raised border around all inlet holes such that HF could be contained in a single region during the etch process. The PDMS etch guide with dam was reversibly contact-sealed to a glass slide. We introduced 10% aqueous HF into the punched inlet reservoirs and applied a membrane vacuum pump (GAST model DOA-P704-AA from VWR, Irving, TX) sequentially to the outlet reservoirs. We etched for a total of 20 min to achieve desired etch depth (5–10 μm). In order to maintain unreacted HF in contact with the glass, we reapplied the vacuum every 1–2 min.

We created inlet and outlet ports in the etched glass substrate by drilling with a 1.1-mm diamond bit in a high-speed rotary tool. The glass surfaces to be sealed were thoroughly scrubbed with a delicate task wipe (Kimwipe brand, Kimberly-Clark, Neenah, WI) and Alconox detergent (Alconox, Inc. White Plains, NY), which was prepared according to manufacturers’ recommendations. The slide and coverslip surfaces to be bonded were then rinsed with a 0.5% Alconox, 0.5% (w/v) calcium(II) acetate hydrate (Sigma-Aldrich) slurry in water. It should be noted that the mixture precipitates at this concentration.

The panes of glass were held close to each other and rinsed with a stream of DI water, which was directed into their interstitial space. This rinsed out all visible slurry. Then, while still held within the stream of water, the two pieces of glass were brought into full contact. The total rinsing time was limited to ~30 s during which we were careful to avoid trapping bubbles. Although the calcium in the slurry is very important to get good yields of well-bonded glass, it does not constitute an adhesion layer.¹¹ The glass was rinsed such that no trapped slurry was visible and only residual adsorbed ions were retained.

Figure 1B shows a schematic diagram of the steps. See Supporting Information for the detailed procedure. The glass assembly was blotted with a delicate task wipe and then dried at 60 °C for 1 h. At this point the glass was not permanently bonded and could be inspected for defects in the seal, which were easily visible as they appear surrounded by Newton rings. If they were detected, the seal was released by sliding a scalpel between the two pieces of glass and the procedure was repeated. When a uniform seal was obtained, the glass assembly was placed between two glass slides, clamped by two binder clips, and placed in a 115 °C oven for 2 h.

Alternative Cations

The bond strength and reliability are much greater with calcium(II) acetate in solution relative to Alconox cleaning alone. We tested the specificity of calcium as the key agent in this improvement by replacing it with magnesium and manganese ions. We repeated the sealing and bonding as above with blank glass substrates and coverslips. The calcium acetate in the slurry was replaced by manganese(III) acetate or magnesium(II) acetate at equivalent molar concentration (30 mM). In order to explore the role of pH in the bonding procedure, we cleaned glass with Alconox and a delicate task wipe, as above, and then treated with a mixture of ammonium hydroxide (10 mM) and calcium acetate (30 mM) in water (in place of the slurry). We tested the bond strength by sliding a scalpel between the corner of the coverslip and the glass slide. Applying this test to strong bonds result in the coverslip breaking, while a weaker water seal allowed the coverslip to be ‘peeled’ away.

Fusion Bonding

An etched substrate made of Schott brand D263T flame-polished borosilicate glass (Precision Glass and Optics, Santa Ana, CA) was sealed to a coverslip-thickness piece of the same material as per the procedure above including treatment with the slurry of Alconox and calcium acetate. The assembly was placed in a programmable furnace between two pieces of polished alumina under ~4 kg of weight. We heated the glass according to the following routine in a programmable furnace (Vulcan 3-550, ESP Chemicals, Tucson, AZ): 100 °C (heat at 0.3 °C/min), 625 °C (heat at 4 °C/min), hold for 1 h, and then cool to 50 °C (cool slower than 2 °C/min).

Characterization of EOF and CE reproducibility

A chip prepared according to the low-temperature procedure above (without fusion bonding) was used to test the performance of the chips for CE. The chip design is shown in Figure 2C. Before use, the surface of the channel system was cleaned with 100 mM sodium hydroxide; it was introduced to the channel system by applying a membrane vacuum pump to a waste well. After 20 min, the channels were then flushed with running buffer (10 mM borate) and allowed to sit for 2 h. To clean the channels at a later time, pressure was applied to the cleaning solution reservoir for 1 min (cleaning solution inlet in Figure 2C is denoted “c”). The cleaning solution reservoir also contained 100 mM sodium hydroxide.

The injection method is adapted from previous reports.¹⁰ Briefly, separation buffer (10 mM borate) was pushed continuously through both the inlet and waste channels using regulated house pressurized air during injection and separation. We used a computer-controlled

solenoid gas valve manifold to apply selectively pressure to the inlets. Sample injection is shown in a stepwise fashion in Figure 3A–F. Figure 3B shows the result of directing a pressure pulse to the sample inlet channel (denoted “s” in Figure 2C). A wave of the sample mixture flowed down the sample inlet channel adjacent to the separation channel inlets. The sample flow was then arrested as shown in Figure 3C. The continuous separation buffer flow flushed residual, uninjected sample away (Figure 3D and E). Voltage was applied with ground to left and negative high voltage to the right, which caused sample to move toward the waste channels as shown in Figure 3F. We initiated data collection with the detector positioned at the outlet of the separation channel shortly before we applied the voltage.

RESULTS AND DISCUSSION

Calcium-Enhanced Contact Bonding Is Sufficient for Many Needs without Thermal Fusion

As water between two extremely clean pieces of glass evaporates, it pulls the two panes into contact until they form a strong seal, which is known in other fields as optical contact.⁴ This glass–glass contact seal follows the use of a concentrated acid cleaning solution,^{7,8,11} such as piranha. We attempted to recreate this effect by cleaning in a boiling solution of hydrogen peroxide and ammonium hydroxide. This procedure, however, produced highly inconsistent results comparable to brief scrubbing with Alconox detergent. Intermittently, the evaporation of interstitial water produced a seal, but the surface coverage was incomplete.

We speculated that the alkaline cleaning solutions leave deprotonated silanol groups that are charged or have unfavorable counterions. Strong acid cleaning procedures left the silanol groups uncharged, avoiding electrostatic repulsion or residual monovalent counterions. We hypothesized that any negatively charged groups on the opposing glass surfaces could be used to our advantage if they were bridged by a divalent cation. We were pleased to find that scrubbing by hand with slurry of Alconox (a basic detergent) and Ca^{2+} (in the form of 30 mM calcium acetate) allowed us to produce a seal between unpolished glass quickly and easily and with high reproducibility. Based on experiments with alternative multivalent cations detailed below, however, our original mechanistic hypothesis seems to be simplistic.

Nonetheless, the results with calcium are reliable and very reproducible. Without heat treatment (or drying temperatures up to 80 °C) this seal is reversible. We refer to this reversible glass adhesion as a water seal. As was noted by Chiem et al.,⁸ this water seal can be separated with a scalpel as long as one piece of glass is thin enough to flex. After calcium treatment and 1 h at 115 °C, however, the seal is effectively permanent. Out of a concerted trial of 36 attempts, 21 were acceptably sealed at the water seal step. In those cases where the seal was imperfect, the pieces of glass could be separated and sealed again with ease. All of the 21 samples that carried forward to the final heat treatment did successfully bond with 100% yield. These results were consistent with those for dozens of chips we made for use in other experiments.

Once treated with heat in this manner, the coverslip cannot be removed without breakage, and we refer to this as a bond. Under intense pressure applied to a fluid inlet, a circular portion of the coverslip beneath the well explodes outward but the glass–glass bond remains intact. Prolonged (overnight) treatment with 0.1 M sodium hydroxide in the channels has no

noticeable effect on the bond. Figure 2A shows a portion of the channel system made with this method. This bond is acceptable for virtually all CE applications; we tested the strength of the bond to 4 kV cm^{-1} without any loss of integrity. For very extreme applications, it may be necessary to use a fusion bond. In that case, the calcium-enhanced contact bond may be used as an intermediate step to increase the yield of thermal fusion when performed in non cleanroom conditions.

Calcium-Enhanced Contact Bonded Glass Can Be Thermally Fused

Figure 2B shows the D263T glass prepared with calcium-enhanced contact bonding followed by fusion bonding. Because of the isotropic nature of glass etching with HF, features broaden in this step. In the specific instance shown, some of the $2\text{-}\mu\text{m}$ channels have broadened into each other. The original pattern had eight straight channels, of which only two were fully successful. We attempted thermal fusion on crown glass slides and coverslips, but breakage virtually always results, presumably due to CTE mismatch. Even with CTE-matched glass, consideration must be given to the temperature ramp schedule in order to achieve fusion without allowing the features to sag; small features are particularly vulnerable to this effect.

HF etching with PDMS etch guides has been demonstrated and characterized previously⁹ in our laboratory including etch profile and surface quality. Previously the size range for this technique was restricted due to the hydrophobic surface properties of PDMS. Pretreatment of the PDMS with APTES solves this problem.

A fully fusion-bonded glass chip¹¹ has been reported to withstand field strengths higher than $100 \text{ kV}\cdot\text{cm}^{-1}$. For most uses, the calcium-enhanced bond would be sufficient (CE can be easily accomplished at voltages below $1 \text{ kV}\cdot\text{cm}^{-1}$). In cases where thermal fusion is necessary (e.g., when extremely high field strengths or high pressures are needed), prebonding with calcium is still advantageous. The D263T can be evaluated at the intermediate water seal step prior to heat treatment, as at this point dust or other defects are correctable. This is a significant improvement compared to our experience with in-house fusion bonding as, after high-temperature treatment, any defect will be permanent.

Cation Specificity of the Bond

We predicted that a divalent cation could bridge opposing negative charges on a base-cleaned glass surface. This was borne out with calcium (as 30 mM calcium acetate) and so we tested the hypothesis with alternative cations. Alconox detergent is primarily composed of anionic surfactants with sodium cations, and on the basis of the low yield of successful sealing or subsequent bonding with Alconox alone, we did not explore further monovalent cations.

A slurry of 0.5% Alconox (w/v) and 30 mM magnesium acetate produced results far inferior to the results obtained with calcium. After treatment at $115 \text{ }^\circ\text{C}$, the result was an impermanent seal over only a portion of the surface. A slurry of 0.5% Alconox (w/v) and 30 mM manganese(III) acetate produced results intermediate between those obtained with calcium and those with magnesium. A permanent bond formed, but the surface coverage was uneven.

We tested the possibility that the Mg^{2+} and Mn^{3+} were relatively unavailable due to higher stability of associations with detergent molecules. We applied 30 mM manganese(III) acetate or 30 mM magnesium acetate to glass separately from the detergent. Neither case produced a bond. To determine if the application of the divalent cation and the alkaline or surfactant solution must be simultaneous, we attempted the bonding procedure with calcium acetate alone (without Alconox) or with ammonium hydroxide to raise the pH. The results of removing Alconox or replacing Alconox in the slurry with ammonium hydroxide were similar. In both cases, the glass did bond successfully as in the simultaneous Alconox/calcium cases. The surface coverage seemed qualitatively less even. Nonetheless, this confirms that calcium is uniquely able to facilitate the bond.

Based on this evidence, an effective bond would seem to be related to something more specific than simple ion-bridging. It may be that soda lime glass tolerates calcium in the bonded structure because it already contains significant amounts of calcium. Calcium might be more receptive to the proposed condensation involved in this kind of bonding.⁷ It may alternatively be due to the relative availability of calcium ions to the deprotonated silanol groups related to its low position in the Hofmeister series.¹⁴ In any case, Ca^{2+} seems unique in allowing for this low-temperature glass bond.

Capillary Electrophoresis Separation and Reproducibility in a Glass Chip with Calcium-Assisted Bonding

Figure 3G shows the linearity of EOF and implicitly the reproducibility of multiple runs on a single chip. The EOF was determined by the retention time of a zwitterionic marker, Rhodamine B. The EOF velocity at various voltages was fit to a line; tens of runs on the single chip produced results consistent with the linear fit to within 4% deviation. The voltages ranged up to $4 \text{ kV} \cdot \text{cm}^{-1}$ after which the chip still produced consistent results at lower voltages. PDMS chips would be inapplicable under these conditions and generally do not produce such repeatable results even at lower voltages. In addition to the EOF marker, the sample mixture also contained FITC-labeled glycine and glutamate. The separation is shown in Figure 3F. Over a separation distance of only 1 cm, we were able to detect four peaks, although in such a short distance the glycine and free dye peaks cannot be fully resolved.

CONCLUSION

The use of Ca^{2+} -assisted bonding removes a key bottleneck in the rapid prototyping of glass microfluidic devices. Our procedure avoids 10-h heating and cooling schedules required for fusion bonding and tolerates mismatches in the CTE of different glass substrates. Furthermore, our technique does not require strong-acid cleaning baths or expensive, polished glass. Our method may be particularly convenient for capillary electrophoresis applications, because glass has a well-defined surface in comparison to other polymers or plastic and thus has a stable and reproducible electroosmotic flow. The ability to apply high field strengths is advantageous in many instances, such as in single-molecule CE¹⁵ and in high-throughput CE-based assays. The ability to fabricate glass chips easily and quickly

with high yield should expand the use of glass chips for many applications that would benefit from the materials properties of glass.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

P.B.A. thanks the NSF for a graduate fellowship. This work was supported by the NIH (EB005197 and NS052637) and the Keck Foundation.

References

1. Harrison DJ, Manz A, Fan Z, Luedi H, Widmer HM. *Anal Chem.* 1992; 64:1926–1932.
2. Wei J, Nai SML, Wong CK, Lee LC. *Thin Solid Films.* 2004; 462–463:487–491.
3. Wu H, Huang B, Zare RN. *Lab Chip.* 2005; 5:1393–1398. [PubMed: 16286971]
4. Greco V, Marchesini F, Molesini G. *J Opt A: Pure Appl Opt.* 2001; 3:85–88.
5. Iles A, Oki A, Pamme N. *J Microfluidics Nanofluidics.* 2007; 3:119–122.
6. Chen L, Luo G, Liu K, Ma J, Yao B, Yan Y, Wang Y. *Sens Actuators, B.* 2006; 119:335–344.
7. Jia ZJ, Fang Q, Fang ZL. *Anal Chem.* 2004; 76:5597–5602. [PubMed: 15362926]
8. Chiem N, Lockyear-Shultz L, Andersson P, Skinner C, Harrison DJ. *Sens Actuators, B.* 2000; 63:147–152.
9. Rodriguez I, Spicar-Mihalic P, Kuyper CL, Fiorini GS, Chiu DT. *Anal Chim Acta.* 2003; 496:205–215.
10. Allen PB, Doepker BR, Chiu DT. *Anal Chem.* 2007; 79:6807–6815. [PubMed: 17676758]
11. Wang HY, Foote RS, Jacobson SC, Schneibel JH, Ramsey JM. *Sens Actuators, B.* 1997; 45:199–207.
12. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu H, Schueller OJ, Whitesides GM. *Electrophoresis.* 2000; 21:27–40. [PubMed: 10634468]
13. Stjernstrom M, Roeraade J. *J Micromech Microeng.* 1998; 8:33–38.
14. Zhang Y, Cremer PS. *Curr Opin Chem Bio.* 2006; 10:658–663. [PubMed: 17035073]
15. Schiro PG, Kuyper CL, Chiu DT. *Electrophoresis.* 2007; 28:2430–2438. [PubMed: 17577880]

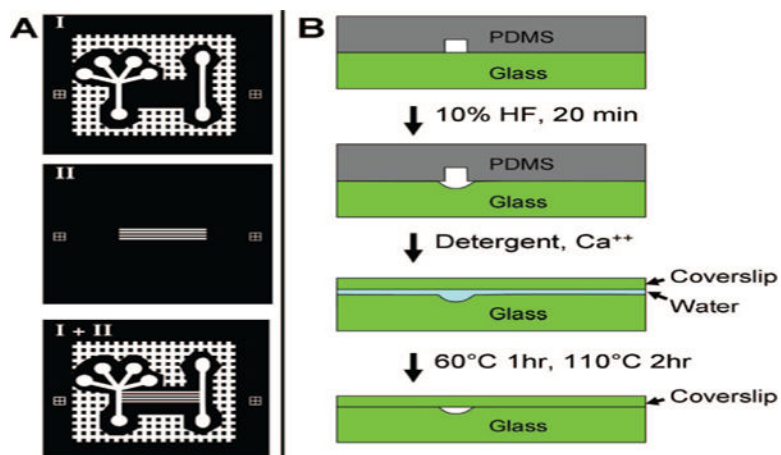


Figure 1.

(A) Sequence from top to bottom showing two masks (I and II) and the combined pattern (I + II) utilized for the photolithographic generation of the master for the molded PDMS etch guide. We implemented this design in two layers to avoid problematical low-aspect-ratio structures in PDMS. (B) A schematic diagram of the major stages of etching and bonding shows how the PDMS etch guide directs HF onto the glass substrate to produce channels. After drilling, the glass is cleaned and rinsed with detergent containing calcium. The glass and its cover are rinsed with water and then assembled wet. The assembly was dried and then baked at 115 °C to produce the final bond.

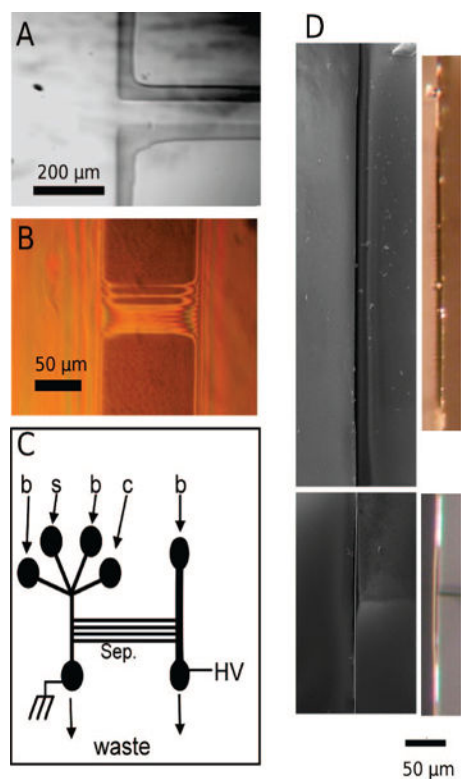


Figure 2.

(A) Images of the final products showing the beveled edges of the wide channels. (B) The effects of broadening on small and dense ($2\ \mu\text{m}$ separated by $4\ \mu\text{m}$) channels due to the isotropic nature of HF etching. (C) The strategy for the use of this design. The labeled arrows show the inlets for buffer (b), sample (s), and cleaning solution (c) as well as the outlets for waste. The electrical connections are also indicated. (D) The side profile of the wide side channels (top) and the more narrow separation channels (bottom). To the left are SEM images of the channel cross sections; light microscope images of the corresponding channels are at right.

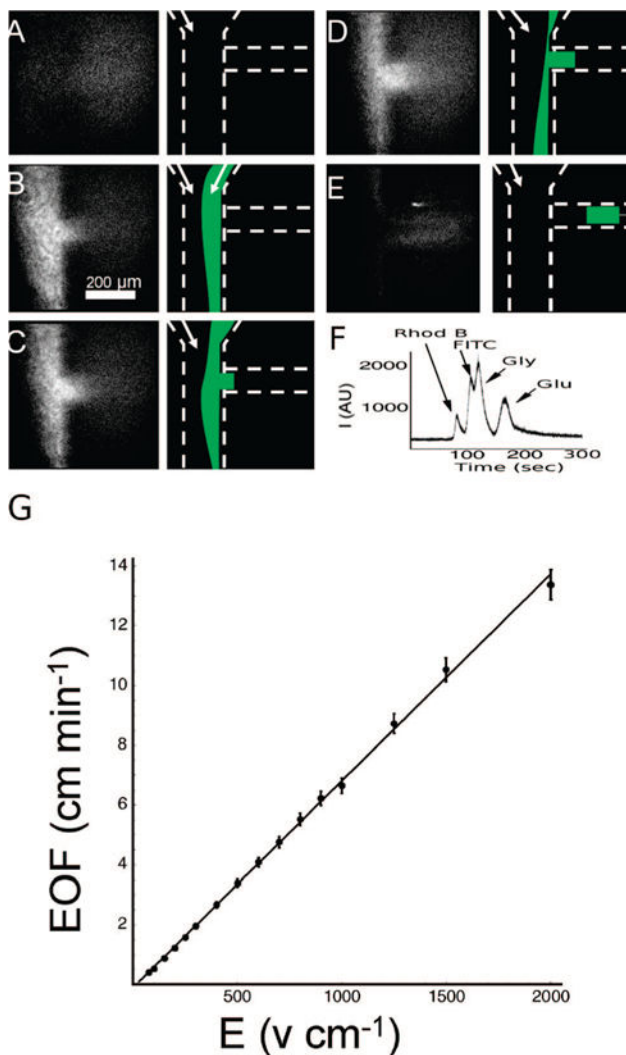


Figure 3. (A–E) Sample injection shown as a sequence of events with fluorescence micrographs at left and a corresponding diagram at right. Arrows in the diagram indicate applied pressure. The sequence starts with (A) an empty channel with pressure directed into the buffer inlet. Pressure is then directed into both the buffer and the sample inlet and sample begins to flow (B) into the channel. Pressure to the sample is removed (C) and the sample flow recedes from the wide channel as buffer flushes it out of the channel. As a result, a small plug of sample is injected into the small separation channel (D). Finally, voltage is initiated (E) and the plug of sample is moved down the separation channel to the right. The electropherogram in (F) shows four peaks, which are labeled as follows: Rhod B for the rhodamine B peak, which also acts as a marker of EOF, FITC for the unreacted dye peak, Gly for labeled glycine, and Glu for labeled glutamate. In the example, the channel was 1 cm long and the applied voltage was 400 V. (G) We performed a series of measurements of EOF based on the retention time of zwitterionic Rhodamine B injected as in (A–F). The results are plotted with

their corresponding best-fit line. The error bars represent the relative standard deviation between runs.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript