2

Fabrication of a Glass Capillary Electrophoresis Microchip With Integrated Electrodes

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Summary

In this chapter, a detailed outline delineating the processing steps for microfabricating capillary electrophoresis (CE) with integrated electrochemical detection (ECD) platforms for performing analyte separation and detection is presented to enable persons familiar with microfabrication to enter a cleanroom and fabricate a fully functional Lab-on-a-Chip (LOC) microdevice. The processing steps outlined are appropriate for the production of LOC prototypes using easily obtained glass substrates and common microfabrication techniques. Microfabrication provides a major advantage over existing macro-scale systems by enabling precise control over electrode placement, and integration of all required CE and ECD electrodes directly onto a single substrate with a small footprint. In the processing sequences presented, top and bottom glass substrates are photolithographically patterned and etched using wet chemical processing techniques. The bottom substrate contains seven electrodes required for CE/ECD operation, whereas the top substrate contains the microchannel network. The flush planar electrodes are created using sputter deposition and lift-off processing techniques. Finally, the two glass substrates are thermally bonded to create the final LOC device.

Key Words: Capillary electrophoresis (CE); electrochemical detection (ECD); Lab-on-a-Chip (LOC); microfluidics; microelectrodes; microfabrication.

1. Introduction

This chapter presents a detailed description of the fabrication steps involved in constructing a microfluidic platform for analyte separation via capillary electrophoresis (CE) including electrochemical detection (ECD) electrodes integrated "on-chip." The objective of this chapter is to provide a detailed processing roadmap that will provide persons familiar with microfabrication technology the necessary information to enter a cleanroom and fabricate a functioning Lab-on-a-Chip (LOC) microdevice. The process steps outlined are

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appropriate for the production of LOC prototypes using traditional glass substrates and general microfabrication manufacturing techniques. Although viable alternative microfabrication techniques exist for the mass production of these devices (e.g., micromechanical machining, micro-molding, micro-embossing), traditional fabrication techniques will be described because these processes leverage 40 yr of success by the integrated circuit industry and, thus, are well characterized and readily available.

2. Device Design

Prior to fabrication, the microfluidic device was designed and modeled using a finite element (FEA) software package (CoventorWare®, Coventor, Raleigh, NC) to determine the appropriate geometry for the channels and the detection electrodes. Previous microcapillary investigations performed by Jacobson and Ramsey ***(1)*** demonstrated that by "focusing" the analyte stream at the intersection of the sample loading and separation microchannel, unwanted diffusion of the analyte from the injection stream into the separation microchannel arm could be eliminated. Unfortunately, accomplishing this with an unbalanced (i.e., unequal arm lengths) microchannel system geometry requires multiple high voltage power supplies, typically one for each terminating capillary arm. To solve this problem, a "balanced-cross" microcapillary pattern was developed and modeled, consisting of four equal length capillary arms (10,000 μm). This greatly simplified the electrical requirements for driving the electrokinetic flow by allowing operation of the system with a single power supply for both injection and separation modes. The details of the supporting electronics is beyond the scope of this chapter and, therefore, will not be addressed here, but a description of the design and development of the power supply and electrochemical detection circuitry has been previously reported ***(2)***. To reduce the "footprint" required by this geometry, each of the two arms that establish the sample loading microchannel were designed with 90° bends (**Fig. 1**). It has been previously demonstrated that bends or turns in a microfluidic channel will distort an analyte plug such that detection can be negatively affected ***(3)***. In this case, the channels that incorporate 90° bends are utilized only during sample loading (also referred to as sample injection); and, therefore, the plug integrity at the intersection is not compromised by either of these bends.

3. Microfabrication

3.1. Photomask Development

After the microchip design process has been completed, the desired channel and electrode configurations are created in a pattern generation software

Fig. 2. (*Opposite page*) L-edit mask pattern for the **(A)** microchannels (channel linewidth = 50 μm) and **(B)** electrodes (working electrode linewidth = 40 μm). The final device design following superposition of the negative of the two masks is shown in **(C)**. In **Fig. 2A**, the circles represent the location of the reservoirs and the square represents

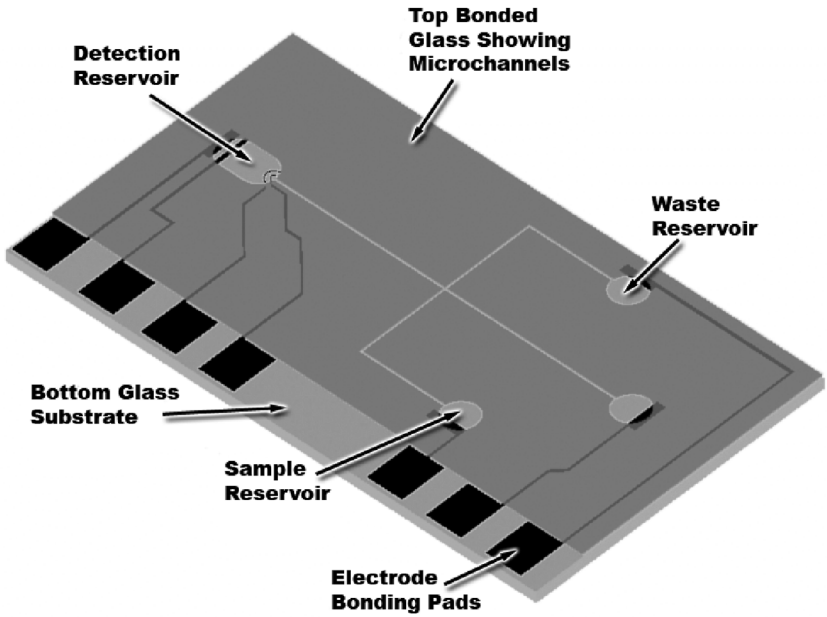
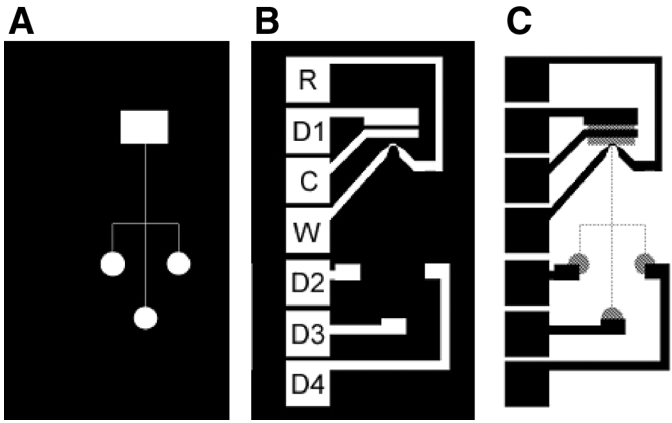


Fig. 1. Schematic of the capillary electrophoresis system developed using finite element modeling software. Highlighted are the four reservoirs (sample, waste, buffer, and detection) and seven patterned electrodes required for operation (four high-voltage electrodes for CE and three amperometric electrodes for ECD). The three amperometric electrodes (working, reference, and counter) are positioned in the detection reservoir such that the working electrode is closest to the microchannel exit ***(4)***.



the location of the larger reservoir required to accommodate the EC detection and CE driving electrodes. In **Fig. 2B**, R = the reference electrode; C = the counter electrode; W = the working/detection electrode; and D1, D2, D3, and D4 are the driving electrodes for the detection, sample, buffer, and waste reservoirs, respectively.

(L-EDIT; Tanner Research, Inc., Pasadena, CA) and saved as a CIF (CalTech Intermediate Form), GDS (graphic design system), DXF (drawing exchange format), or PS (postscript) file. Examples of the patterns developed for the microchannels and electrodes to be discussed are shown in **Fig. 2**. The L-EDIT file is used to generate the master set of photomasks required for the micromanufacturing of the prototype devices. Depending upon the resolution desired for the final device, the photomask set can be fabricated either on glass via an optical or laser pattern generator or on transparency sheets (such as Mylar®) using a high resolution image setter, such as those found at a local print shop (*see* **Note 1**). The major advantage of glass photomasks fabricated using an optical or laser pattern generator is the quality in the line resolution, where linewidths of 1–2 μm can generally be achieved. However, photomasks fabricated with optical and laser pattern generators are expensive because these systems typically cost between $150,000 and $300,000, and the sensitized chrome-covered photomask glass blanks are also relatively expensive. Several organizations provide mask generation services, which typically charge $300–500 per mask, as compared with the lower resolution Mylar photomasks, which typically cost between $15 and $50 per sheet. The two most significant advantages of the Mylar photomask approach are low cost and short turnaround time, both of which are important issues for research and development and rapid prototyping applications. However, the advantages of using the transparency solution are often outweighed by the reduction in the quality of the line resolution, which at best is approx 10 μm.

3.2. Photolithography

Soda-lime glass was chosen as the primary substrate material because it is commercially available, can be processed using traditional microfabrication techniques, and naturally possesses the surface characteristics needed for the requisite wall–buffer charge interface (ζ-potential), thereby eliminating the necessity for any chemical modification of the capillary wall surfaces. This

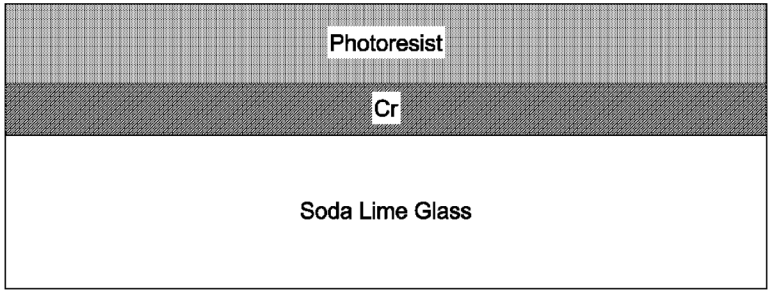


Fig. 3. Photomask blank prior to patterning.

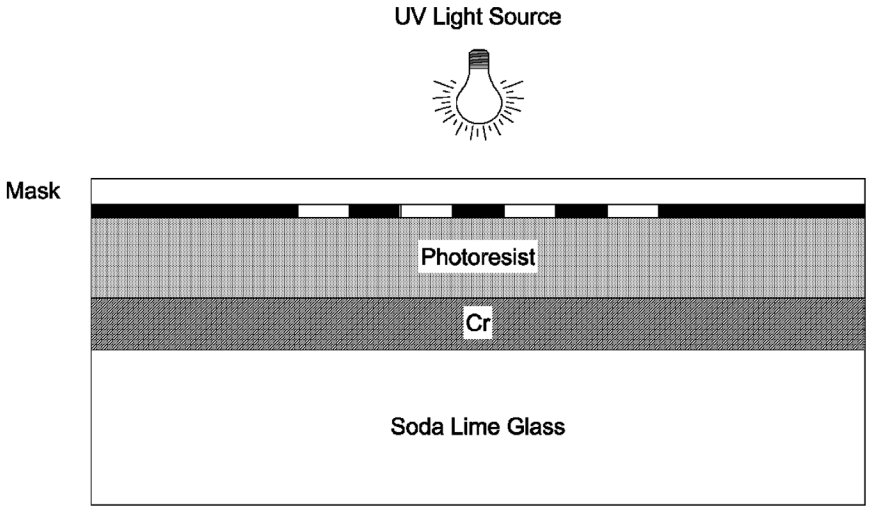


Fig. 4. Schematic of bottom substrate photoresist layer upon exposure to ultraviolet light.

native potential is a fundamental requirement for establishing the electroosmotic bulk flow of the buffer fluid in response to an applied electric field.

One of the easiest and cheapest methods for etching a detailed pattern into glass is to simply use an unexposed photomask blank as the starting substrate material. The photomasks used in this process are 60 mil (1524 μm) thick ultra-flat, soda-lime glass precoated with a low reflective chrome and positive resist (supplied by Nanofilm, Inc.; **Fig. 3**). These photomask blanks become the actual glass substrates, and the ultra-flat surface of the photomask allows the resolution and integrity of the fabricated features to be maintained. The combination of these two properties is critical in the final bonding process.

3.3. Bottom Substrate Processing

The bottom substrate (5 × 3.5 cm) contains the required seven electrodes for CE/ECD operation. These electrodes are formed by a photolithographic liftoff process, bulk micromachining, and sputtering as follows (*see* **Note 2**):

1. The electrode pattern is first transferred onto the precoated positive resist layer using the appropriate darkfield photomask (**Fig. 2B**) and contact lithography (**Fig. 4**). The photomask blank is positioned with the patterned mask in direct contact with the photoresist. The photoresist is exposed to G-line ultraviolet (UV) light from a Mercury arc lamp with a dose of 150 mJ/cm^2 to allow the resist to be patterned as described in **step 2**.
2. The exposed photoresist is then removed with MF-319 developer (Shipley Co., LLC, Marlborough, MA), which is a dilute tetramethylammonium hydroxide (TMAH) solution, to reveal the underlying chrome layer (**Fig. 5**). It is essential

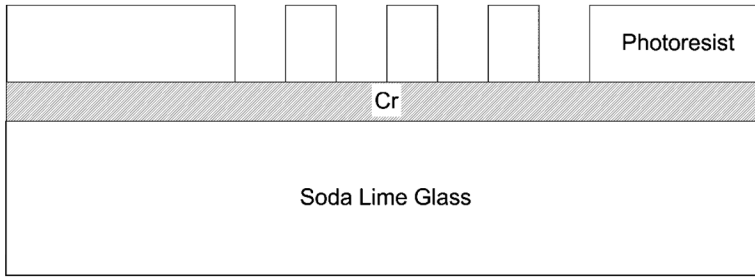


Fig. 5. Schematic of the patterned photoresist layer on the bottom substrate.

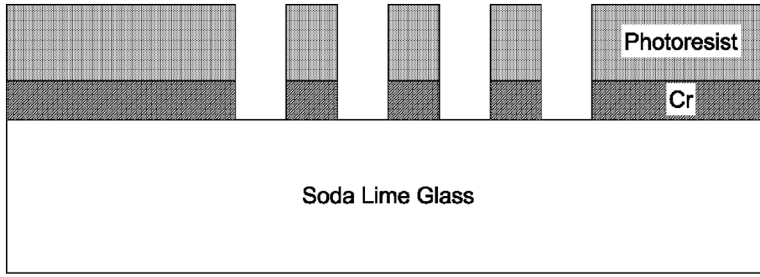


Fig. 6. Schematic of bottom substrate following removal of chrome layer.

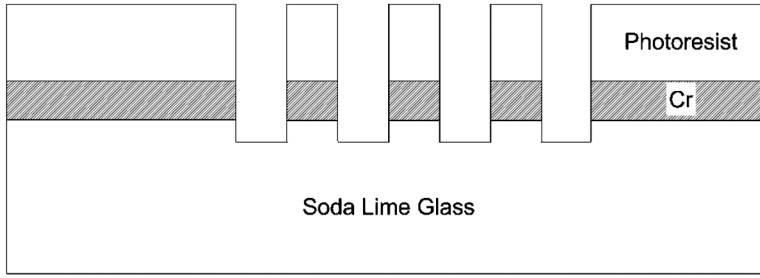


Fig. 7. Schematic of etched recessions in glass substrate before electrode deposition.

that the unexposed photoresist remains on this piece of glass for the liftoff patterning of the Ti/Pt electrode layer described next.

3. The exposed chrome layer is then patterned by a short etch in CEP-200 Micro Chrome etchant (Microchem, Inc., Newton, MA). The final product is a soda-lime glass substrate with a patterned layer of photoresist and chrome, revealing the glass substrate in the desired electrodes regions only (**Fig. 6**).
4. After lithography and chrome etching, the exposed glass is etched for 30 s in a 6:1 buffered oxide etch (BOE; J.T. Baker, Phillipsburg, NJ) to form approx 0.3-µm recessions (**Fig. 7**). This short etch produces shallow recesses that allow the subsequent sputter-deposited metal layer to lie flush or just below the original glass surface, which is critical for keeping the final surface of the bottom substrate planar; otherwise, electrodes protruding above the surface would hinder the

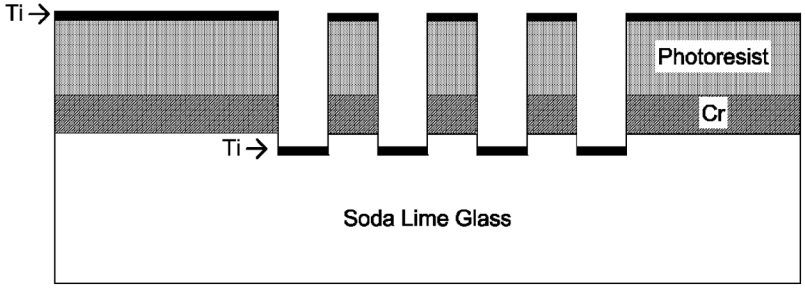


Fig. 8. Schematic of Ti deposited layer.

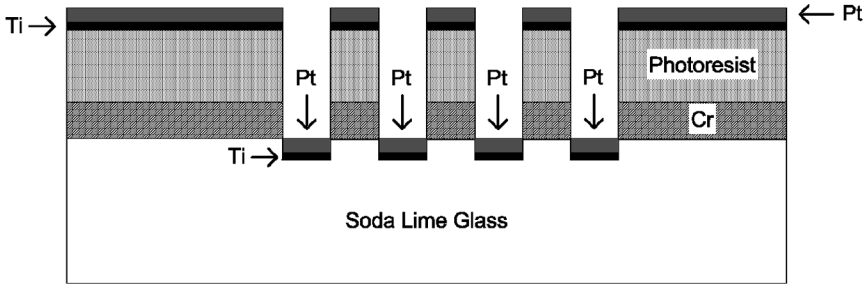


Fig. 9. Schematic of Pt deposited layer.

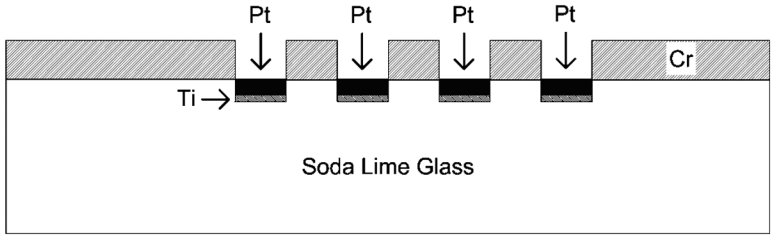


Fig. 10. Schematic of bottom substrate after lift-off process.

bonding process. BOE is a solution of ammonium fluoride and hydrofluoric acid that etches the exposed soda-lime glass at a rate of approx 600 nm/min at room temperature. Following this step, the sample is removed from the solution, rinsed in deionized (DI) water, and dried with nitrogen.

5. A Technics sputtering system with both DC and RF sputtering heads is used to deposit a 10-nm adhesion layer of titanium via RF sputtering (pressure = 10 mTorr; power = 300 W; t = 0.5 min) followed by DC sputtering of 300 nm of platinum (pressure = 10 mTorr; power = 300 W; t = 13 min) over the entire glass substrate (**Figs. 8** and **9**).
6. Patterning of the electrode material is accomplished by soaking the substrate in acetone, which causes the underlying positive photoresist layer to dissolve (**Fig. 10**). As this protective resist layer is removed in the solvent, the Ti/Pt electrode material located above it is "lifted off." The electrode material remains

anchored to the glass substrate only in the exposed recessed patterns in the glass substrate. This liftoff process allows the dual-composition electrodes to be patterned in a single processing step as opposed to wet etching, which would require multiple selective etching steps.

7. The final process is the removal of the remaining chrome layer in the nonelectrode regions. The original chrome layer which was once protected by the liftoff resist is removed by a second etch in CEP 200 Micro Chrome etchant (**Fig. 11**). The exposed Pt electrodes are unaffected by the selective chrome etch. This substrate only requires final cleaning before it is ready to be bonded to its companion top glass substrate that will contain the necessary etched channels and reservoirs (described in **Subheading 3.4.**).

3.4. Top Substrate Processing

Fabrication of the top glass substrate (5 × 2.5 cm) is slightly more straightforward. This substrate contains the microcapillaries and reservoir openings

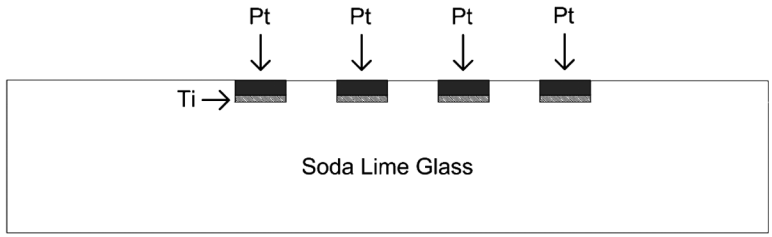


Fig. 11. Schematic of completed bottom substrate with recessed electrodes.

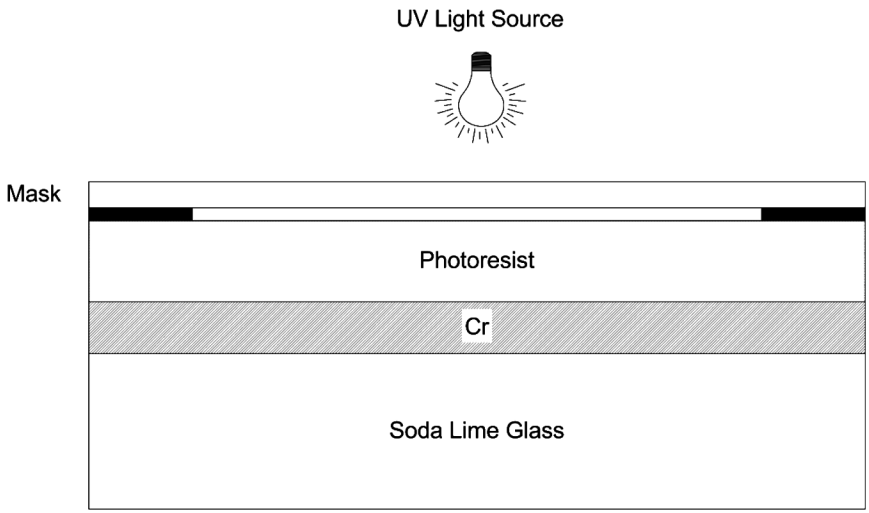


Fig. 12. Schematic of top substrate patterning with ultraviolet light.

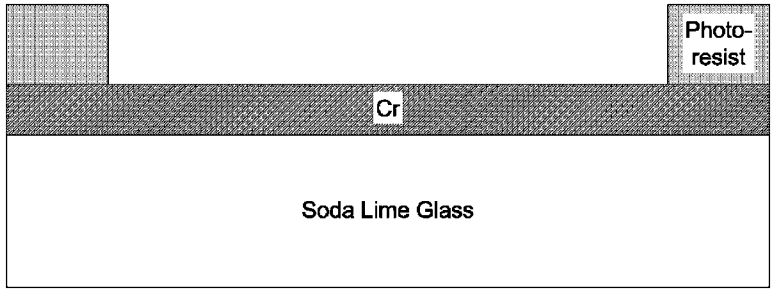


Fig. 13. Schematic of top substrate following removal of the photoresist layer.

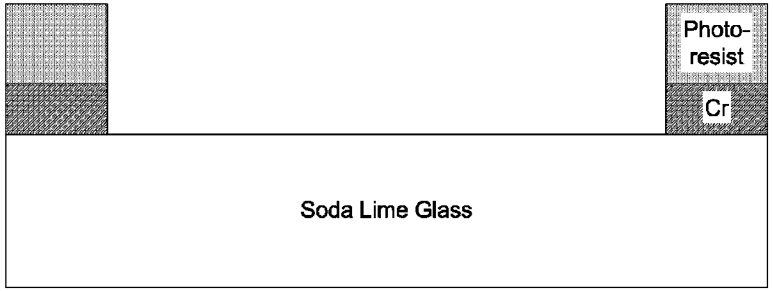


Fig. 14. Schematic of top substrate following removal of chrome layer.

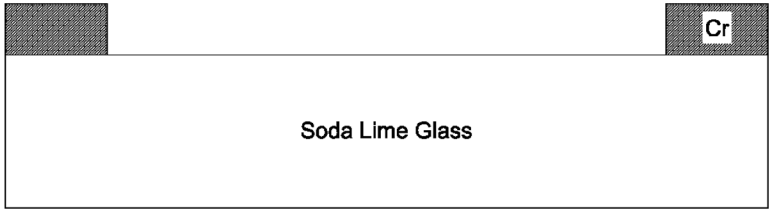


Fig. 15. Schematic of top substrate following removal of the final photoresist layer.

and is formed by a combination of photolithography, bulk micromachining, and conventional glass-drilling.

1. The microchannel pattern is transferred onto the precoated positive resist layer of a second ultra-flat soda-lime photomask blank (Nanofilm, Inc., Valley View, OH) using the appropriate darkfield photomask (**Fig. 2A**) and contact lithography (**Fig. 12**). The photomask blank is positioned with the patterned mask in direct contact with the photoresist. The photoresist is exposed to G-line UV light from a Mercury arc lamp with a dose of 150 mJ/cm^2 to allow the resist to be patterned.
2. The exposed photoresist is then removed with MF-319 developer (Shipley Co.) to reveal the underlying chrome layer (**Fig. 13**).

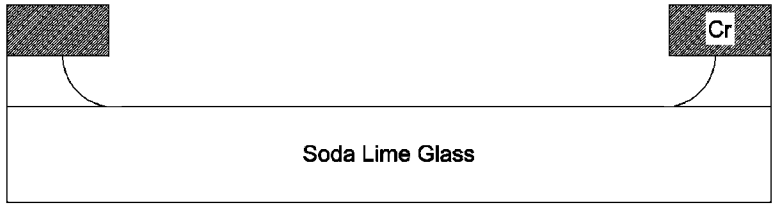


Fig. 16. Schematic of top substrate following glass etching.

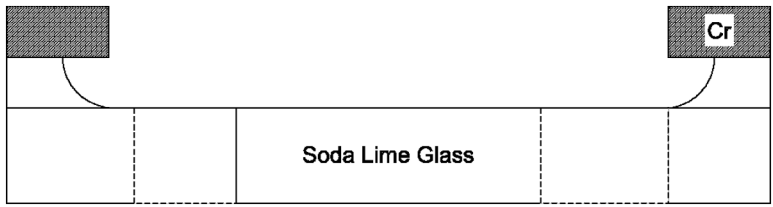


Fig. 17. Schematic of top substrate following reservoir drilling.

3. The exposed chrome layer is then etched using CEP-200 Micro Chrome etchant, providing access to the glass substrate only in the desired microchannel regions (**Fig. 14**).
4. Although it is useful to keep the photoresist on the bottom glass substrate during electrode formation, it is best to remove the resist prior to etching the top microchannel glass substrate. Therefore, the top substrate is placed in acetone to remove most of the photoresist; and the remaining organic residue is removed by a short dip in Nano-Strip, which is a mixture of sulfuric acid and hydrogen peroxide (Cyantek, Fremont, CA). It is important to rinse the Nano-Strip coated substrate by first placing it in a beaker of DI water and then rinsing under running DI water for 1 min. Rinsing the Nano-Strip coated substrate directly under running DI water with a layer of Nano-Strip remaining on the substrate may cause a loss of the patterned chrome layer, which is the only layer remaining to act as a mask during the glass etching step (**Fig. 15**).
5. Once dried with nitrogen, the 20-μm deep channels are etched in the top glass substrate by placing the substrate for 1 min in 6:1 BOE (**Fig. 16**). Once etched, this glass must be rinsed thoroughly in DI water, dried with nitrogen, and inspected under the microscope. If a crazed formation is seen in the etched glass regions, the glass substrate should be discarded and the procedure started over since the crazes in the glass will potentially result in irregular fluid flow presumably owing to interruption of the ζ-potential at the fluid–wall interface. If the glass looks smooth but has many small, black fragments around the perimeter of the channels, then the etching process has begun appropriately. The dry glass substrate should then be dipped in Nano-Strip and rinsed in a beaker of DI water, followed by a rinse under running DI water and again dried with nitrogen. Finally, the top glass substrate is etched in BOE for 29 min, followed by the same rinsing, nitrogen drying, dipping in Nano-Strip, rinsing, and nitrogen drying process previously described (*see* **Note 3**).

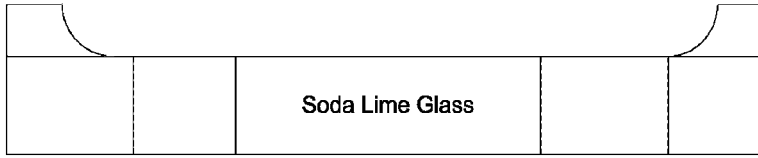


Fig. 18. Schematic of top substrate following removal of the chrome layer.

6. With the final etch width criteria of the channel met (80 µm for the top of the channel; 50 µm for the bottom of the channel) and depth (20 µm), the four reservoir openings (d = 5 mm) are then drilled into the top glass substrate at the positions of the terminal ends of each capillary arm (**Fig. 17**) using a Dremmel® tool fitted with a diamond core drill bit. These predrilled holes serve as the buffer, sample, waste, and detection reservoirs. An extra hole is drilled approximately one radius away from the center of the original detection reservoir hole to accommodate a slightly larger detection reservoir. This can be done as a possible preventive measure to minimize the gradual background current increase during amperometric detection because of the buildup of unreacted analyte after successive CE separations.
7. The final processing step involves the removal of the remaining chrome layer using CEP 200 Micro Chrome etchant (**Fig. 18**). The top glass substrate requires only a final cleaning before it is bonded to the bottom glass substrate containing the electrode patterns.

3.5. Glass-to-Glass Bonding

Prior to bonding, the bottom glass substrate containing the electrodes and the top glass substrate containing the microchannels are dipped in Nano-Strip, rinsed with DI water in a beaker and then rinsed under running DI water, and finally dried with a nitrogen gun. An RCA1 base clean is prepared by heating five parts DI water and one part 40% ammonium hydroxide in a beaker to 70°C. Once at 70°C, the heat is turned off, and one part 30% hydrogen peroxide is stirred into the solution. The microchannel glass substrate is submerged in the solution for a minimum of 5 min. The electrode glass substrate is added to the solution for a short soak of no more than 1 min (to minimize adhesion damage to the electrodes). Both glass pieces are rinsed under running DI water for 2 min; the contacting faces of the electrode substrate and the channel substrate are brought together under running DI water. Although water is allowed to remain between the two joined pieces of glass, the outer regions of the glass are dried with nitrogen. A well-joined microchip will have just enough water between the two glass substrates so the substrates will not slide freely (*see* **Note 4**). The electrodes and channels are aligned manually under a stereo zoom optical microscope with the adhesion of the water maintaining the alignment during transit to the furnace (*see* **Note 5**).

The assembled microchip is placed on an alumina tile, and the top of the microchip is covered with a second alumina tile. An additional steel block

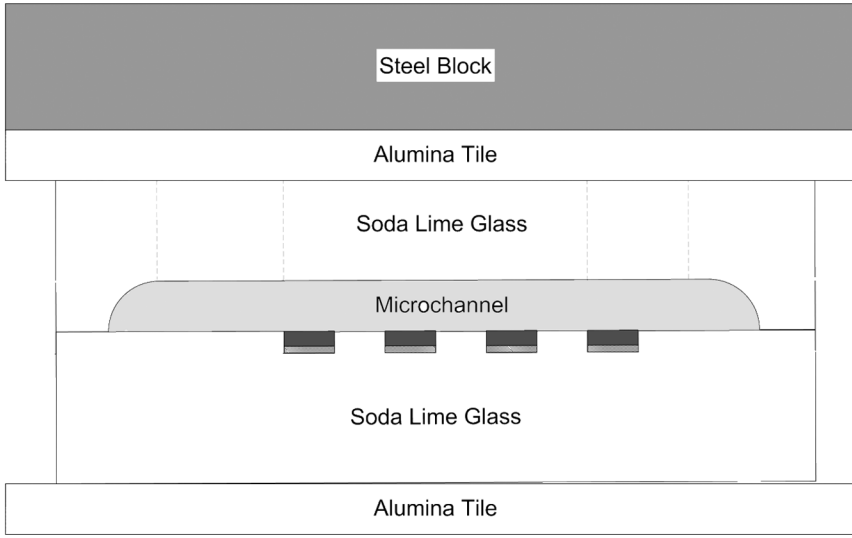


Fig. 19. Schematic of bonding "stack" prior to insertion in furnace for thermal bonding.

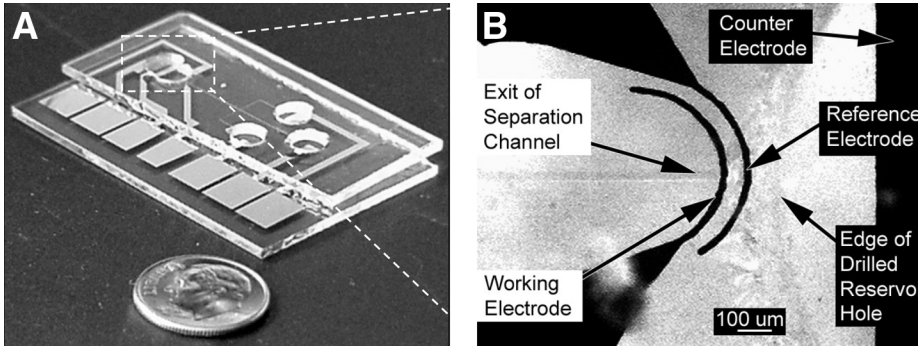


Fig. 20. Images of the **(A)** final microfabricated capillary electrophoresis/electrochemical detection device with integrated Pt electrodes and thermally bonded micromachined glass substrates and (**B**) ×40 magnification of the detection reservoir containing the electrochemical detection electrodes.

(weight) is added to the top alumina tile to provide a 50-g/cm^2 pressure on the two glass plates (*see* **Note 6**). The "stack" assembly is carefully placed in a well-controlled tube furnace for thermal bonding (**Fig. 19**). The bonding sequence involves heating the substrates to a maximum temperature of 625°C at a ramp rate of 3°C/min. The stack is allowed to dwell at this temperature for 30 min in an air environment and is then removed using furnace gloves. With the proper safety clothing and face shield, the alumina tiles and microchip are pried apart. The microchip and alumina blocks are returned to the furnace

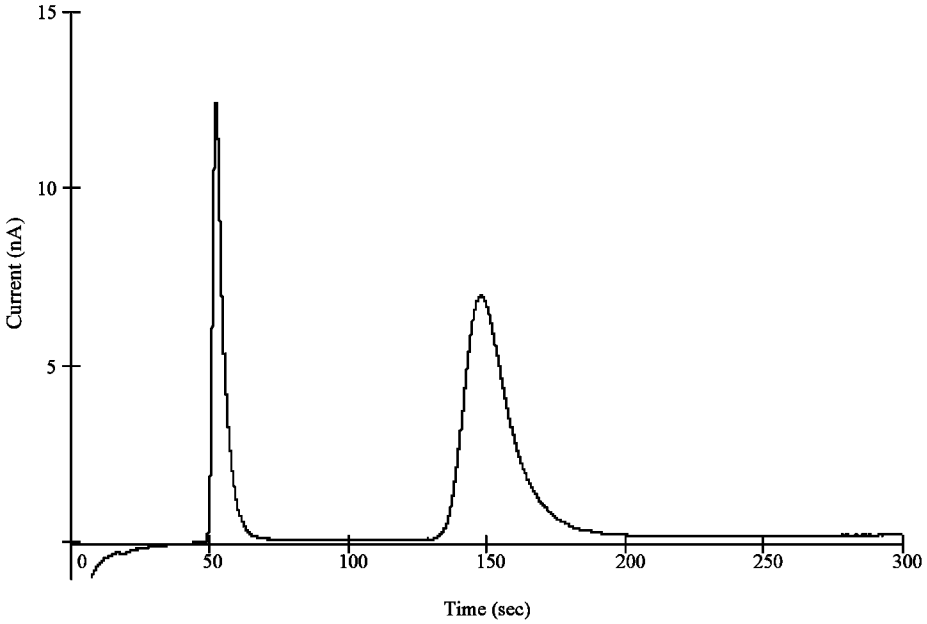


Fig. 21. Electropherogram from the capillary electrophoresis–electrochemical detection Lab-on-a-Chip device for the separation and detection of dopamine (1.1 m*M*) and catechol (2.3 m*M*). Conditions: CE voltage, 200 V; EC potential, +0.75 V vs Pt wire.

without the steel block (load mass) and allowed to cool at a ramp down rate of 3°C/min. Careful ramping of the furnace is required in order to keep the bonded glass assembly from fracturing because of induced thermal stresses. The chip is removed when the furnace reaches 100°C.

3.6. Capillary Electrophoresis Microchip With Integrated CE/ECD Electrodes

Using the previously described process, a functioning CE microchip with integrated CE and EC detection electrodes is manufactured (**Fig. 20**). Such devices have been utilized for hundreds of electrochemical detection experiments typically involving the detection of the neurotransmitters dopamine and catechol (**Fig. 21**), as well as tagged DNA-related compounds. The device has been successfully characterized and has been found to yield reproducible results over a 4-mo time period with little-to-no decrease in performance ***(4)***. It takes approx 1 wk to fabricate a device once the master photomask set has been generated. Cleanliness is critical during all stages of the manufacturing process, and it is especially important to minimize surface contamination during the thermal bonding process. Our research group has produced dozens of LOC prototypes

in this fashion, and the manufacturing process has proven to be both reliable and reproducible.

4. Notes

1. Print shops typically require the PS format.
2. **Figures 3–19** are not drawn to scale.
3. The process of etching, drying, and nanostrip cleaning may need to be repeated a number of times to maintain etch quality.
4. Occasionally there is so little water that the chips cannot be moved for alignment. If this occurs, the chips can be placed under running DI water so they can be separated and placed together again for an additional alignment attempt.
5. A final press of the top chip to the bottom chip will keep the two halves from moving with respect to each other during transit.
6. Important to use a steel block and/or weight with an overall surface area equal to or greater than the overall footprint of your chip.

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