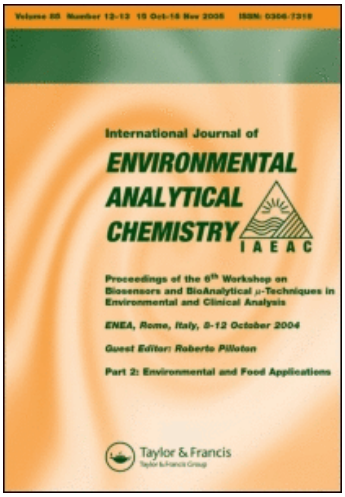


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

# Glass microchannel technology for capillary electrophoresis

Lucanos M. Strambini[a]; Massimo Piotto[b]; Andrea Nannini[a]
[a] Dipartimento di Ingegneria dell'Informazione, Università di Pisa, Pisa, Italy [b] IEIIT-Sezione di Pisa, CNR, Pisa, Italy

**To cite this Article** Strambini, Lucanos M. , Piotto, Massimo and Nannini, Andrea(2005) 'Glass microchannel technology for capillary electrophoresis', International Journal of Environmental Analytical Chemistry, 85: 9, 681 — 686
**To link to this Article: DOI:** 10.1080/03067310500146144
**URL:** http://dx.doi.org/10.1080/03067310500146144

*Intern. J. Environ. Anal. Chem.*
Vol. 85, Nos. 9–11, 10 August–15 September 2005, 681–686

Taylor & Francis
Taylor & Francis Group

# Glass microchannel technology for capillary electrophoresis

LUCANOS M. STRAMBINI*†, MASSIMO PIOTTO‡
and ANDREA NANNINI†

†Dipartimento di Ingegneria dell'Informazione, Università di Pisa,
Via G. Caruso–56122, Pisa, Italy
‡IEIIT-Sezione di Pisa, CNR, Via G. Caruso–56122, Pisa, Italy

(*Received 8 October 2004; in final form 14 February 2005*)



The fabrication process of glass chips for capillary electrophoresis by means of micromachining is reported. The device is made up of two glass substrates joined by means of thermal fusion bonding. Selective wet etchings were used to define five microchannels, four for samples injection and one for the separation, while the access holes were obtained with diamond drills. The fabrication process required only one photolithographic step and the thermal fusion bonding did not reduce the uniformity and integrity of the channels. Good results in terms of microchannels shape definition, repeatability and glass surface quality have been obtained.

*Keywords:* μ-Systems; Capillary electrophoresis; μ-Fluidics; Glass micromachining

## 1. Introduction

The crossover of microfabrication technology from the integrated circuit industry into analytical chemistry has led to a considerable increase in the number of integrated microfluidic devices, known as micro total analysis systems (μTAS) or lab on a chip (LOC), which are able to perform analytical functions required by an analysis. Among them, chips for chemical analysis have been considered one of the most promising due to their short analysis times, high separation efficiency, high sample throughput, minute samples and reagents consumption and easy automation [1, 2]. In these applications glass is the preferred material due to its good optical properties, well-known surface characteristics and high breakdown voltage [3]. Unlike silicon, the micromachining of glass is not well standardized, especially about the etching mask and glass bonding where many different technological solutions have been proposed [4–8].

In this work, we present the fabrication process of a device for capillary electrophoresis made up of two 800 μm thick microscope glass slides. Five microchannels, four for samples injection and one for the separation, have been fabricated on a substrate using a selective wet etching. Access points to the channel reservoirs have

*Corresponding author. Fax: +39-050-2217522. Email: lucanos.strambini@iet.unipi.it

*International Journal of Environmental Analytical Chemistry*
ISSN 0306-7319 print: ISSN 1029-0397 online © 2005 Taylor & Francis
DOI: 10.1080/03067310500146144

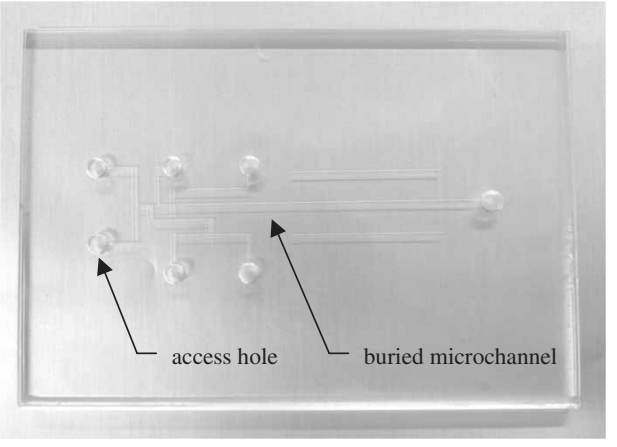


Figure 1. Optical photograph of the device highlighting the access point to the buried microchannels.



been provided by 1 mm holes, made in the second substrate by means of a diamond drill. The two substrates have been finally joined by means of the fusion bonding technique. Differently from other works reported in the literature [4–6, 9, 10], we propose a simpler fabrication process developed on ordinary cheap microscope slides. The etching mask is a thin photoresist/chromium double layer and the fusion bonding is based on a single step at 620°C for 1 h without the need of weights on the samples. In figure 1 an optical photo of the device is reported.

## 2. Fabrication process

The integrated microfluidic device is schematically shown in figure 2. As it can be seen, the structure is made up of five microchannels: the longest one (20 mm) is provided for the separation while the others are for samples injection and are 9 mm long. The channels width was 20 μm in the mask while at the end of the process was about 80 μm at the top and 30 μm at the bottom, as shown in figure 5.

In order to fabricate buried glass microchannels, the bottom substrate, with the etched microchannels, and the top substrate, with the access points to the corresponding channel reservoirs, were assembled by means of the fusion bonding technique. The substrates were two $26 \times 40\,mm^2$ microscope slides.

The fabrication process, schematically drawn in figure 3, can be synthesized into three main steps: (1) micrometric channel definition on the bottom glass substrate by means of selective glass etching, (2) fabrication of channel reservoir access points on the top glass by means of drilling, (3) assembling of the two substrates by a fusion bonding technique.

The microchannels pattern was defined on the glass substrate with a selective wet etching using a double layer as mask: a metal hard mask, chromium, and a polymeric soft mask, photoresist. The use of a double layer as mask was mandatory to have a highly resistant and chemically stable mask, able to withstand the subsequent HF-based etch step. After a preliminary step of substrate cleanliness with acetone, ethanol and deionized water in an ultrasonic bath, to increase the adhesion of the

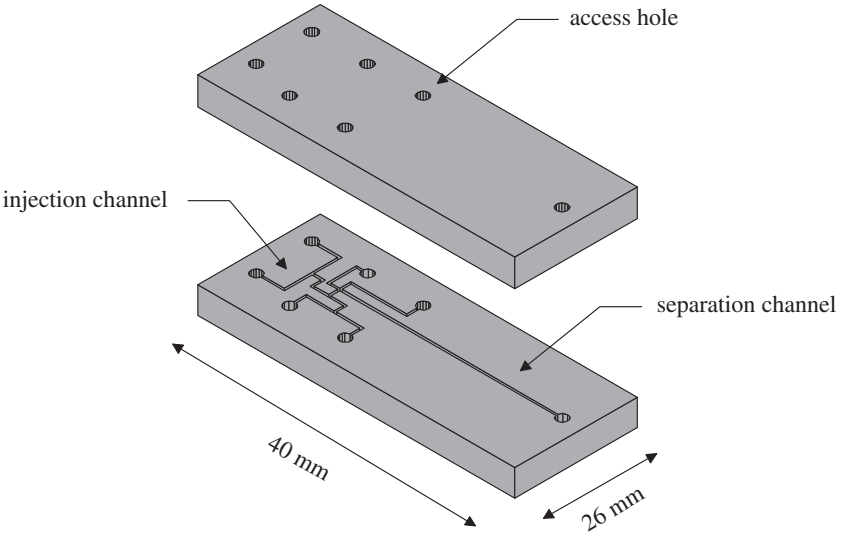


Figure 2. Schematic view of the device (not to scale).



metal mask, a 800 nm thick chromium layer was deposited by means of e-beam evaporation at a residual pressure of $1.4 \times 10^{-6}$ Torr, substrate temperature of 280°C and deposition rate of $1.5\,\text{Å}\,\text{s}^{-1}$. Chromium thickness was chosen as a compromise between the necessity of having a mask thick enough to support the wet etching of the glass substrate, and the need of a thin layer to limit the chromium under-etching.

After a second cleaning cycle with acetone, ethanol and deionized water in an ultrasonic bath and a dehydration at 200°C for 60 min in a convection oven, a 1500 nm thick positive photoresist (Microposit S1818) layer was spun on the glass substrate (figure 3a). The microchannel pattern was defined with a standard contact photolithography using a 1 μm resolution mask aligner (Mask Aligner MJB-3, Karl Suss). Finally, to increase the resistance to the following chemical etching, the photoresist layer was submitted to a thermal treatment, in a convention oven, with a higher temperature (130°C) and longer time (45 min), than those usually used in a post-baking treatment.

Chromium etching was performed using a 150 g ceric ammonium nitrate ($(NH)_4Ce(NO_3)_2$), 100 mL perchloric acid (60% $HClO_4$), and 1000 mL $H_2O$ mixture for 2 min at room temperature (figure 3b).

The bottom glass substrate was etched with a hydrofluoric acid (48% HF), nitric acid (65% $HNO_3$) and deionized water mixture (1 : 1 : 5 by volume) [11, 12] at a temperature of 38°C with an etching rate of $4.5\,\mu\text{m}\,\text{min}^{-1}$ (figure 3c). The double layer mask allowed to achieve etch depths of about 25 μm without detectable defects on the glass surface. The etching of the back side of the substrate was avoided by protecting it with a sacrificial glass slide, glued with a proper wax (Buehler Crystalbond 509) soluble in acetone. The resist/metal mask was finally removed, respectively, in acetone and in chromium etching solution (figure 3d).

In figure 4 an SEM photo of the intersecting point between the separation channel and an injection channel is shown. The good quality of the glass etching and lithographic definition allowed us to obtain a geometrically well-defined sample injection volume. This property is mandatory to perform high-resolution electrophoresis analysis

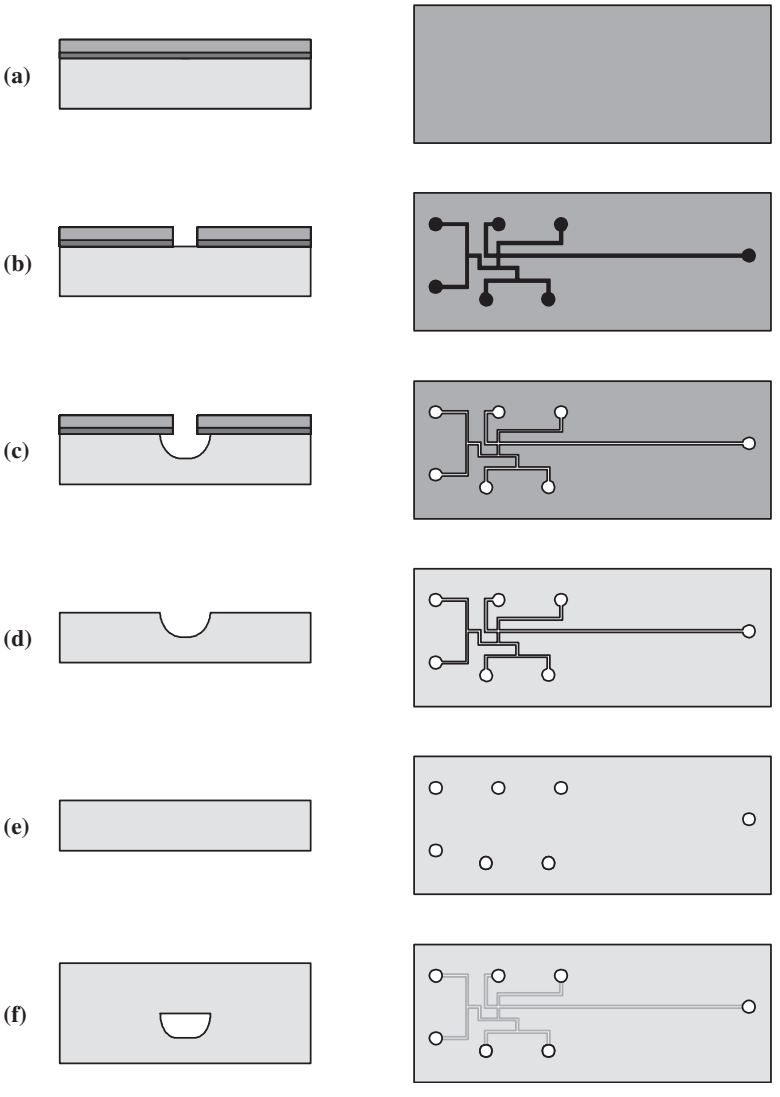


Figure 3. Schematic drawing of the main steps of the fabrication process: cross-section (left), plan view (right).

using electro-osmotic pumping in a valveless configuration [13]. As can be seen in figure 4, a good smoothness of the channel walls was obtained with the employed etching solution, similar to that obtained by Stjiernström and Roeraade [14] with a bufferd HF/HCl mixture. The irregular edge of the channel, visible in figure 4, was the consequence of an adhesion problem of the chromium mask during the glass wet etching which caused only a slight roughness of the upper surface of the side walls.

In order to provide an access point to the channels reservoirs, 1 mm holes were drilled in the top glass substrate by means of diamond drills (figure 3e). Holes with sharp edges were obtained by assembling two sacrificial microscope slides on both side of the glass substrate with a proper wax.

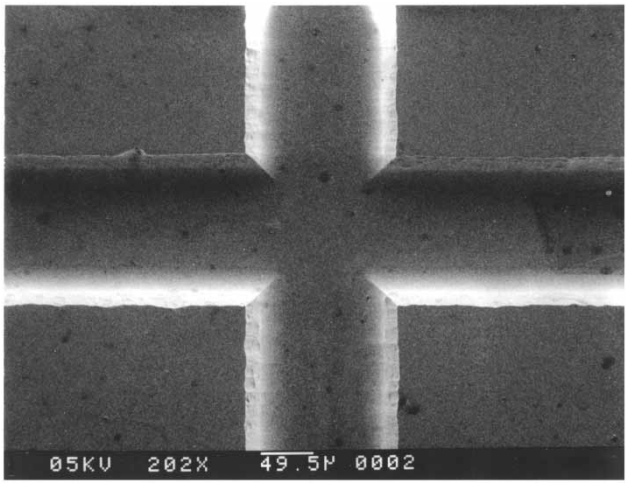


Figure 4. SEM photograph of microchannel intersection after glass etching.



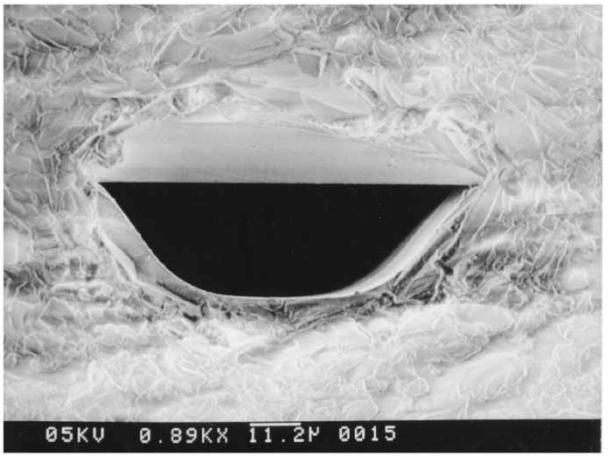


Figure 5. SEM photograph of the microchannel cross-section at the end of the fabrication process.

Both substrates, the bottom one and the cover, were carefully cleaned in a sulfuric peroxide solution at 120°C for 20 min and by successive washings with acetone, ethanol and deionized water in ultrasonic bath. This cleaning step is very critical because the fusion bonding yield is heavily dependent on the cleanliness of the two surfaces to be joined.

After drying in nitrogen flow, the two glasses were manually joined, so that the drilled holes were aligned to the channel reservoirs, and put into the muffle over a polished ceramic plate. This plate was used to avoid any glass surface defects due to the roughness of the internal muffle floor. For the fusion bonding the following thermal cycle was used: 1 h ramp from room temperature to 620°C, storage at 620°C for 1 h and then overnight cooling down. In figure 5 a cleaved cross-section of a microchannel with a depth of 25 µm after the fusion bonding is shown: the absence of a separation surface

between the two glasses confirms the good quality of bonding. This result was obtained using a more simple thermal cycle than others reported in literature [9, 10] and avoiding the employ of weights placed on the glasses during the bonding. In fact, weights produced defects on the top glass surface even with an interposed polished ceramic sheet. As far as the bonding yield is concerned, only small unbonded areas were present in some samples after the thermal cycle and usually far from the channels where they did not compromise the chip functionality. Besides, the reiteration of the thermal treatment did not substantially improve the bonding quality in disagreement with literature data [9, 10]. This discrepancy is probably due to the fact that we did not put any weights over the glasses in these repeated thermal cycles.

As far as the cross-section shape is concerned, a non-perfect semi-circular wall was obtained as shown in figure 5. This tapered profile is due to poor adhesion of the chromium layer to the glass surface [15].

## 3. Conclusion

A fabrication process for capillary electrophoresis devices based on glass micromachining has been presented. Glass chips have been fabricated by means of selective glass etching with an $HF/HNO_3$ solution and fusion bonding with a simple thermal cycle. The device is made up of two $26 \times 40\,mm^2$ microscope slides with a thickness of 800 μm and has five microchannels, four for samples injection and one for the separation. Good results in terms of smoothness of the channels walls, sharpness of the intersecting points between the channels and bonding yield have been obtained.



## Acknowledgement

This research is financially supported by the project FIRB 'Microsystems for Genetic Diagnostics'.

## References

[1] L. Zhang, F. Dang, Y. Baba. *J. Pharm. Biomed. Anal.*, **30**, 1645 (2003).
[2] A. Manz, N. Graber, H.M. Widmer. *Sensors and Actuators B*, **1**, 244 (1990).
[3] V. Dolnik, S. Liu, S. Jovanovich. *Electrophoresis 2000*, **21**, 41 (2000).
[4] A. Grosse, M. Grewe, H. Fouckhardt. *J. Micromech. Microeng.*, **11**, 257 (2001).
[5] M. Ilie, V. Foglietti, E. Cianci, A. Mintoti. *Proceedings of SPIE*, **5227**, 318 (2003).
[6] Zhonghul, H. Fan, D.J. Harrison. *Anal. Chem.*, **66**, 177 (1994).
[7] N. Chiem, L. Lockyear-Shultz, P. Andersson, C. Skinner, D.J. Harrison. *Sensors and Actuators B*, **63**, 147 (2000).
[8] T. Ito, K. Sobue, S. Ohya. *Sensors and Actuators B*, **81**, 187 (2002).
[9] J.C. Roulet, R. Völkel, H.P. Herzig, E. Verpoorte, N.F. de Rooij, R. Dändliker. *J. Microelectromech. Syst.*, **10**, 482 (2001).
[10] Z.H. Fan, D.J. Harrison. *Anal. Chem.*, **66**, 177 (1994).
[11] G.A.C.M. Spierings, J. Van Dijk. *J. Mater. Sci.*, **22**, 1869 (1987).
[12] G.A.C.M. Spierings. *J. Mater. Sci.*, **26**, 3329 (1991).
[13] D.J. Harrison, P.G. Glavina, A. Manz. *Sensors and Actuators B*, **10**, 107 (1993).
[14] M. Stjiernström, J. Roeraade. *J. Micromech. Microeng.*, **8**, 33 (1998).
[15] G.A.C.M. Spierings. *J. Mater. Sci.*, **28**, 6261 (1993).