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TOPICAL REVIEW

Lab-on-chip technologies: making a microfluidic network and coupling it into a complete microsystem—a review

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Abstract

Microfluidics is an emerging field that has given rise to a large number of scientific and technological developments over the last few years. This review reports on the use of various materials, such as silicon, glass and polymers, and their related technologies for the manufacturing of simple microchannels and complex systems. It also presents the main application fields concerned with the different technologies and the most significant results reported by academic and industrial teams. Finally, it demonstrates the advantage of developing approaches for associating polymer technologies for manufacturing of fluidic elements with integration of active or sensitive elements, particularly silicon devices.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

A heart attack is a typical situation for which a fast diagnostic is of paramount importance. In addition to an electrocardiogram, a blood test is often necessary, that is conventionally done in a laboratory. With the portable system marketed by the Biosite company, this diagnostic can be made in only 10 min, directly with the patient [1]. The drop of blood is simply deposited in a reservoir in the system that then performs the entire test: minute quantities of blood are displaced and injected into several tiny chambers in which different reactions take place.

This example clearly illustrates the principle of a lab-on-chip: integrating all the functions of a human-scale test laboratory including transferring samples, drawing off a precise volume of a chemical product, mixing with reagents, heating, titration, etc, on a system of a few square centimetres.

The first essential need for the manipulation of small quantities of liquid or gas fluids is 'micro-plumbing', in other words a network of microchannels with dimensions typically between 10 and 100 μm. The first demonstrators were made from glass or silicon, thus benefiting from the wide experience in micro-engineering acquired through microelectronics, and more generally in the field of microsystems [2, 3]. However, there is no doubt that microfluidics is becoming more democratic and much more widespread, making use of PDMS moulding technologies introduced in the middle of the 1990s [4, 5]. The extreme simplicity and the impressive robustness of this process enable scientists from all backgrounds to get involved in the adventure without needing high technology facilities. There is no doubt that the reason why the microfluidics field has become so wide ranging and has reached its present popularity is its multidisciplinary nature, as can be seen in figure 1 that shows the increase in the annual number of scientific publications listed in the ISI-Web of Knowledge database [6].

However, a lab-on-chip is not simply a network of microchannels. It also includes other functions depending on the application such as pumps, valves, sensors, electronics, etc. Therefore, it can be considered as a complex microsystem including mechanical, electronic, fluid functions, etc. In this framework, monolithic silicon integration processes have drastic disadvantages: an excessive cost due to large surface areas (compared with an integrated circuit) necessary for the

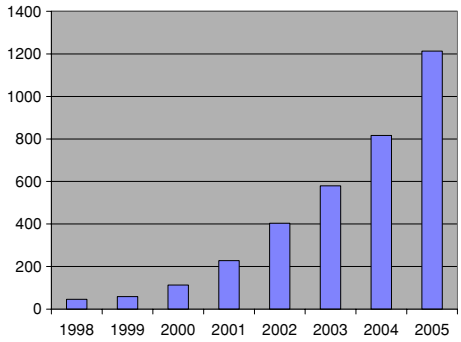


Figure 1. Number of publications per year including the term 'microfluidics', according to ISI-Web of Knowledge database.

microfluidic network and an electrical incompatibility with the high voltages involved in electrokinetic applications.

Adaptation of polymer moulding techniques at the micro and even nanometric scales (casting of PDMS, hot embossing of PMMA, etc) [7–9] has developed very strongly over the last 10 years for manufacturing of simple fluidic networks, but integration into a complete microsystem is far from being trivial. At the moment, there is still a challenge to couple these different existing structured microfluidic parts together, to integrate a sensor or an actuator at any level, or to transfer the fluidic part into a microsystem with precision.

The purpose of this review is to provide a general overview of the state-of-the-art of technological tools available for manufacturing a microfluidic network, varying from a simple purely fluidic 2D network to a complex 3D network integrating the most advanced functions. This review includes a brief remainder about microtechnologies, and then discusses silicon and glass systems as well as polymer-based processes. It also presents manufacturing examples in metal or even ceramic, before discussing the challenge of system integration. The second part presents different applications illustrating these manufacturing techniques.

2. Microtechnologies for microfluidics

Since the 'planar' technology was introduced into microelectronics at the end of the 1950s, micromanufacturing techniques have never stopped improving, continuously extending the limits of miniaturization. Silicon was initially chosen for its semi-conducting properties and the excellent quality of its oxide, and was also used to develop mechanical functions including resonant beams, micro-motors, etc [10–12]. The concept of microsystems (MEMS—Micro-Electro-Mechanical System in the United States, MST—MicroSystem Technologies in Europe) emerged during the 1980s at the University of Berkeley as the integration of the different functions of a complete system on a single chip [13]. The first example of a microsystem marketed by Analog Devices in 1993 was the ADXL50 accelerometer that consisted of a capacitive sensor accompanied by its associated electronics monolithically integrated onto a 10 mm^2 substrate [14].

MEMS technology takes advantages from microelectronic production tools that allow for the manufacturing of thousands of miniaturized objects in

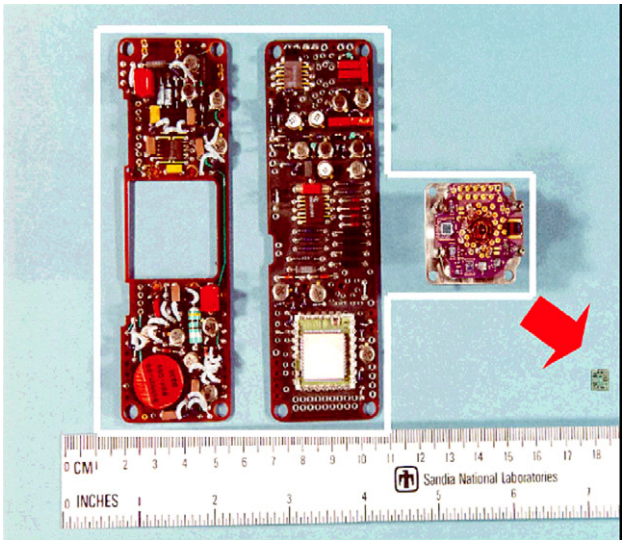


Figure 2. Comparison between an accelerometer constructed using traditional technologies and microsystems: the chip includes the same functions as the complex assembly [18], © 2003, with permission from Elsevier. Also used with the permission of Sandia National Laboratories.

parallel and thus that reduces drastically manufacturing costs. Systems are also much smaller and much more reliable due to integration and elimination of connections between components as illustrated in figure 2. Finally, miniaturization provides a means of performing new functions that cannot be done in any other way, for example such as switching of optical signals by micromirrors [15–17].

Several examples of microsystems are now used in daily life: accelerometers that are present in modern cars (airbag trigger system) [19, 20], and also in Nike shoes (integrated pedometer), Sony Playstation games consoles (measurement of the inclination of the joystick) and GPS systems [21, 22], inkjet print heads [23–26], arrays of micromirrors in video projectors [27, 28], etc. Many sectors are now concerned such as optical and radio frequency telecommunications (switches, variable capacitances and inductances) [29–31]. Finally, the interest in microsystems in the chemistry and biomedical fields has never ceased increasing, particularly since the development of the miniaturized total chemical analysis system (μTAS) concept by A Manz at the beginning of the 1990s and then since the development of labs-on-chip [32].

Over the last 10 years, this popularity has been a driving force for the development of new types of microsystems combining electrical and mechanical functions with microfluidic functions (microwells, microchannels, valves, pumps). It also encouraged the introduction of new processes and materials in microtechnologies. The glass technology widely used at first, combining wet etching and thermal bonding is limited by difficulties in terms of integration and aspect ratio. Although etching of silicon is much more developed, particularly with deep reactive ion etching, its incompatibility with the strong potentials imposed in capillary electrophoresis (CE) that is at the heart of many labs-on-chips, and with electrokinetic pumping that is also widely used, is a serious disadvantage that prevents its use. Apart

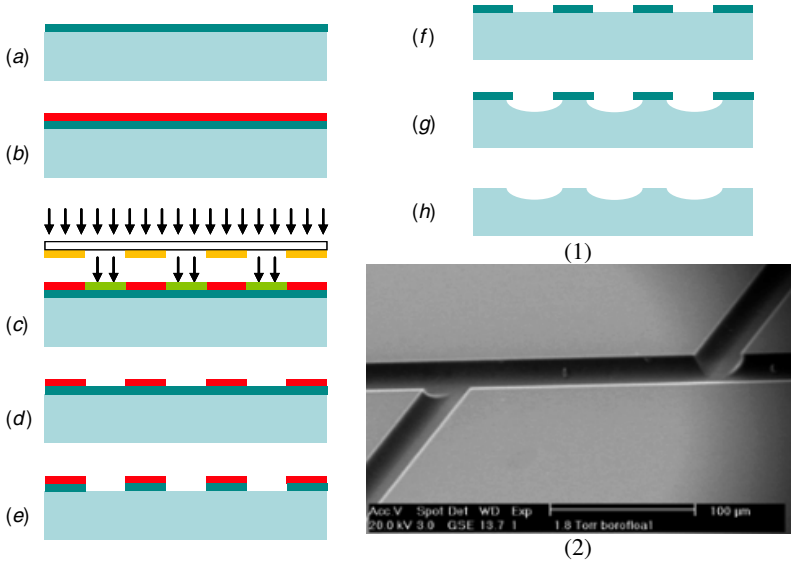


Figure 3. (1) An example of bulk micromachining: wet etching of glass; (2) microchannels etched by wet etching in glass [IMT Neuchâtel] [33] (© 2003 IEEE).

from the gain in cost terms, microtechnologies have become more versatile by opening up to polymers, and due to the wide choice of available properties. The process is also becoming more flexible, for example with micromoulding techniques or obviously the use of thick photoresists such as SU-8, that both avoid the etching step that is expensive and/or using aggressive chemicals. Photolithography resists also have advantages in integration that will be demonstrated later in this chapter. The use of plastics and polymers more generally is now a strong component of micromanufacturing and opens up new prospects in terms of cost, integration and performance by making a complete new range of materials with a very wide variety of properties accessible.

The purpose of this first part is to describe the state-of-the-art of the different technologies used for manufacturing of a microfluidic network and to discuss its integration into a system. The second part describes the advantage of miniaturization and different aspects of flow in a microchannel, and gives a few applications of fluidic micro-systems.

2.1. Silicon and glass technologies

2.1.1. Bulk micromachining. Bulk micromachining refers to the situation in which patterns are defined in the substrate itself. The example of wet etching of glass is shown in figure 3. It clearly illustrates the generic approach to bulk machining. After the substrate has been cleaned, the material that will be used for protection during the etching step is deposited (*a*). A photoresist is coated using a spin coater (*b*). This resist is exposed to ultraviolet radiation through a mask with transparent and opaque areas (*c*). The resist is then developed (*d*) and exposed areas are eliminated (positive resist, as illustrated in figure 3) or remain (negative resist), depending on its polarity. The protective material is etched (*e*) and the resist is removed from the substrate (*f*). The next step is the glass etching step (*h*). Finally, the mask material is removed from the substrate (*f*).

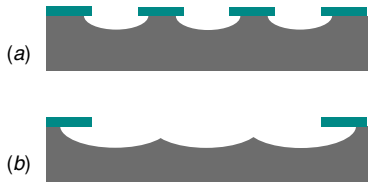


Figure 4. Under-etching phenomenon during isotropic wet etching.

Wet etching. The etching step is fundamental in bulk micromachining [34, 35]. Etching as presented in figure 4 is isotropic, i.e. the etching rate is the same in all directions. Typical examples are wet etching of silicon in a mixture of hydrofluoric acid, nitric acid and ethanol [36], or wet etching of glass in hydrofluoric acid [37, 38]. Isotropic wet etching has several disadvantages, including difficulty in controlling the profile due to the strong influence of stirring and isotropy that severely limits the possible resolution and depth, as illustrated in figure 4

Anisotropic wet etching of silicon [39–42] in solutions such as potassium hydroxide (KOH) or tetramethyl ammonium hydroxide (TMAH) [43–45] can be used to create patterns defined by crystallographic planes of silicon as illustrated in figure 5. This process, together with etching-stop techniques (geometric, electrochemical, implantation of boron) [46–48] makes it easy for manufacturing very thin membranes or suspended structures. However, some simple shapes such as a circular hole cannot be made, the etching profile limits the integration density and the chemicals used are relatively aggressive.

Dry etching. In dry etching techniques [50, 51], the substrate is exposed to an ionized gas. Two major dry etching phenomena are usually considered and are illustrated in figure 6. The physical component is equivalent, at the atomic scale, to sandblasting in which the sand is replaced by inert species such as argon, helium or neon in a ionized form. In

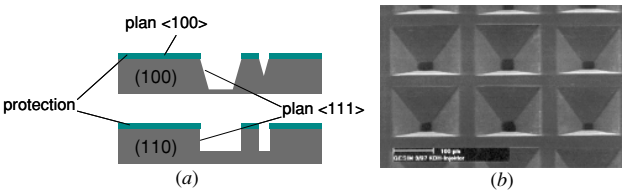


Figure 5. (*a*) Different anisotropic wet etching profiles in single-crystalline silicon; (*b*) examples of microwells made by anisotropic wet etching [GESIM] [49], © 1999, with permission from Elsevier.

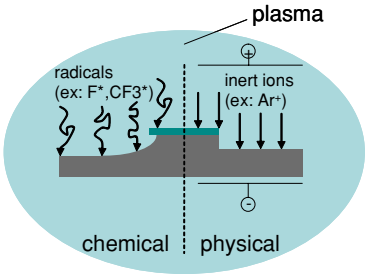


Figure 6. Physical and chemical components in dry etching.

practice, inert ions are generated in plasma and are accelerated by the electric field created between two electrodes. The substrate is fixed to the cathode. This etching is anisotropic but is also relatively slow and is not selective.

The chemical component is the result of the interaction between radicals generated in a plasma from a reactive gas (SF_6, CF_4, ...) and the surface of the substrate. In this case, there is often competition between the deposition and etching. The different parameters of the process (temperature, pressure, concentration of the different gases, etc) will make one of the other phenomena predominate. This chemical etching process provides a means of achieving faster rates and has good selectivity, but is isotropic.

One alternative to these two types of dry etching is reactive ion etching (RIE) that combines the advantages of physical and chemical etching by using reactive ions accelerated by an electric field. Reactive ionic etching is a means of achieving higher etching rates, good selectivity and excellent anisotropy. The deep reactive ion etching (DRIE), advanced silicon etching (ASE) and the Bosch process [52] are now widely used in bulk micromachining and appeared in the middle of the 1990s. As shown in figure 7, each cycle in the process begins with a uniform deposition of a passivation layer (*a*). Deposition of this fluorocarbon type layer is assisted by plasma from a C_4F_8 gas. In the second step (*b*) of the cycle, the passivation layer is etched by plasma formed from SF_6. Strong polarization of the substrate makes the etching anisotropic; in other words the polymer layer is etched more quickly perpendicular to the substrate than through its edges. Finally, the bottom of the trench that is not protected by the passivation layer is etched isotropically (*c*). By repeating this cycle, the trenches can be etched in silicon with excellent aspect ratios. The profile shown in (*d*) is typical of this cyclic process. Unlike anisotropic wet etching, this technique is independent of the crystalline orientation.

SCREAM. Other processes such as the SCREAM (single crystal reactive ion etching and metallization) technology

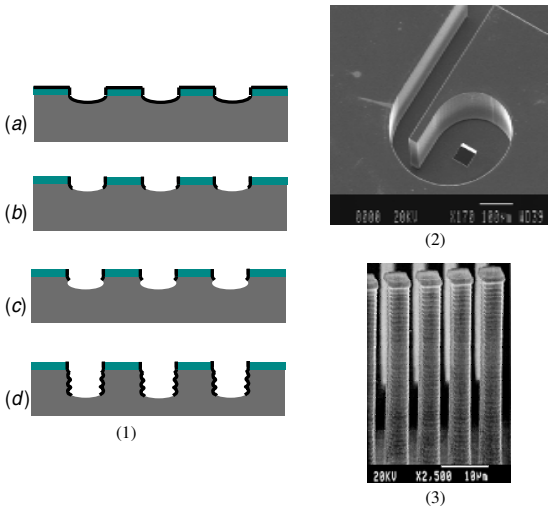


Figure 7. (1) Deep reactive ion etching process; (2) a fluidic vortex diode machined by DRIE [LAAS] [53]; (3) effect of the passivation/etching cycle on a profile [KTH] [54], © 2000, with permission from Elsevier.

invented at the University of Cornell in 1993 sequentially mix anisotropic and isotropic dry etching as illustrated in figure 8 [55, 56]. The process begins on a silicon substrate (*a*) in which openings (*b*) are defined. The silicon is then subjected to anisotropic dry etching (*c*), and a silicon oxide layer is then deposited (*d*), once again structured by anisotropic dry etching which leaves the sides protected (*e*). Finally, isotropic dry etching is done on the silicon to release the structures (*f*) and the substrate is metallized. This technique can be used to obtain suspended structures made of monocrystalline silicon with high aspect ratios.

Assembly. The latter two processes can be used to make suspended structures, but not closed structures directly. An assembly step between two machined substrates is usually necessary to close structures. Several types of bonding techniques have been developed for different applications. These bonding techniques are summarized in figure 9. One of the main concerns is the thermal stress, mainly in the case of heterogeneous assembly, i.e. assembly of materials with different thermal expansion coefficients. The required level of cleanness may also be an obstacle in a process such as thermal bonding. Adhesive bonding is a low-temperature process and does not require complex cleaning procedures but introduces a supplementary material into the structure which may be undesirable in microfluidics in which control of surface properties is critical. It is often estimated that assembly is frequently the most expensive step in microsystem processes.

Microfluidic system manufacturing using bulk micromachining. Bulk micromachining of silicon was derived directly from the integrated circuit industry, and was widely used in the first microfluidic systems. The first example, 11 years before the introduction of the microTAS concept, is the gaseous phase chromatography system developed by Terry *et al* at Stanford at the end of the 1970s and that already integrated a 1.5 m long microchannel, a valve and a detector

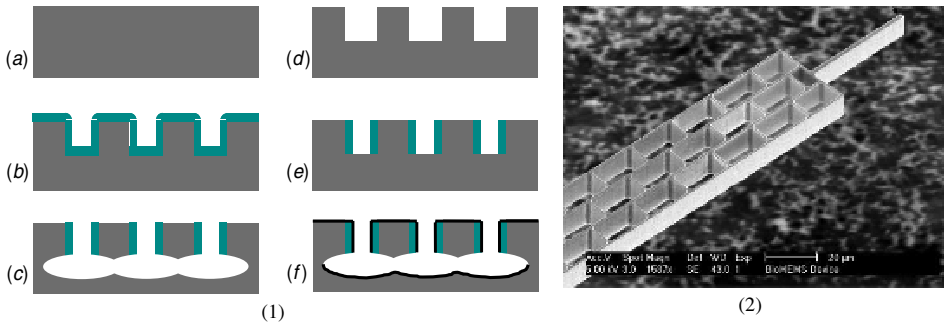


Figure 8. (1) SCREAM process; (2) probe machined by the SCREAM process and used to measure deformation of cells under mechanical stress [University of Illinois] [57], © 2005, with permission from Elsevier.

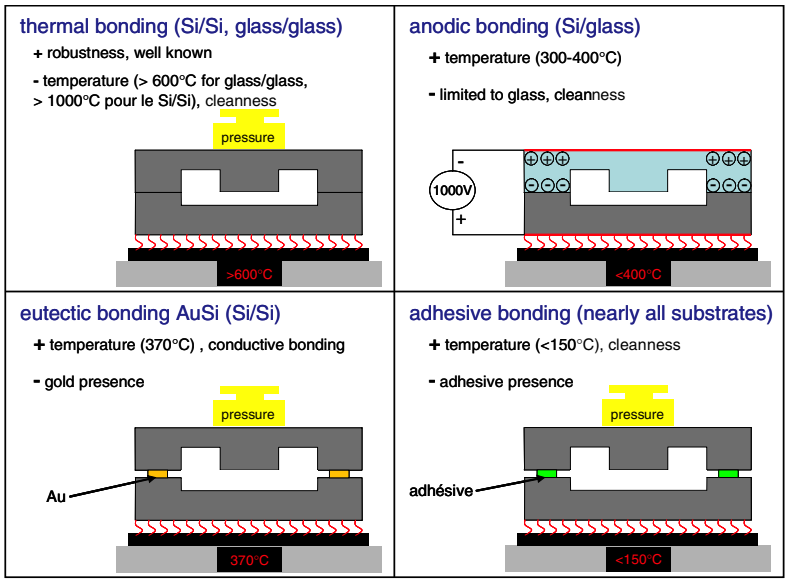


Figure 9. Different types of bonding between substrates.

on a 2 inch silicon wafer (figure 10) [2]. The column was wet etched and was sealed by adhesive bonding with a glass substrate.

Harrison *et al* used anisotropic etching of silicon associated with anodic bonding at the beginning of the 1990s, to make one of the first electrophoresis-on-chip systems [3]. Unfortunately, in this case there is a limitation to the silicon technology. The electrical fields required for CE, which is a separation technique widely used in labs-on-chip as will be mentioned later, are in the range of 100–500 V cm^{-1} and the length of the column is typically a few centimetres, which imposes potentials of the order of a thousand volts. This means that an insulating layer has to be deposited on the silicon. However, the potentials applied are incompatible with the maximum feasible thickness of layers. For example, the breakdown voltage of 1 μm thick layer of good quality silicon oxide does not exceed 720 V. Despite this limitation, DRIE combined with anodic bonding is widely used for other applications due to the reliability of the process and the quality of the structures obtained. Nanofluidic structures have also been made by associating wet anisotropic etching and thermal bonding [58].

Another disadvantage of silicon in microfluidics is its opacity in visible wavelengths, which can be a problem, for example when observing flows or for an external detection. Glass has better optical and electrical properties and has been widely used. The most frequent technology used at the moment is wet etching. Masking is done more frequently with a layer of gold on a chromium adhesion layer but it may also be done for example with a polycrystalline silicon layer, or directly with a resist [59–61]. Assembly is usually done by thermal bonding. It can also be done by anodic bonding, for example when the polycrystalline silicon used as the mask is conserved or by adhesive bonding when components sensitive to temperature or to the cleaning procedure used in thermal bonding must be integrated (electrodes, organic layers) [62]. Companies such as Micronit, LioniX or Micralyne market microfluidic circuits of this type (figure 11(*a*)) [63–65]. As mentioned above, there are limitations to geometries that can be produced using wet glass etching, and the technology remains relatively complex. Other etching techniques such as powder blasting, laser ablation or plasma etching have been studied as shown in figure 11(*b*), but they induce high roughnesses, and are slow or can only be used for limited geometries [66–70]. Nanochannels, in other words channels in which one of the dimensions is less than 100 nm, integrating polysilicon electrodes have been made using methods including RIE of glass and anodic bonding [71].

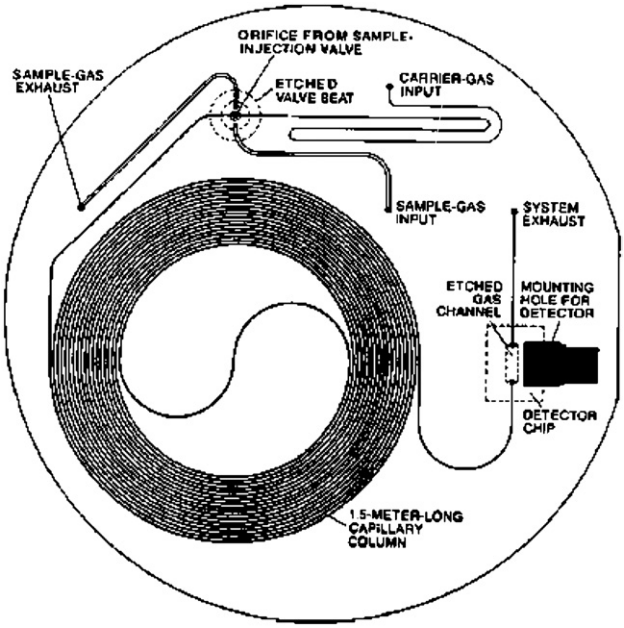


Figure 10. Gas phase chromatography made on a 2 inch wafer [Stanford] in 1979 [2] (© 1979 IEEE).

Photosensitive glasses such as FOTURAN made by Schott, can be used to obtain straight sides but they are expensive and their processing is difficult [72–74].

An alternative technique combines the advantages of silicon etching and the optical and electrical properties of glass. It can be used to make miniaturized transparent insulating channels (μTIC). As described in figure 12, it begins with etching of silicon using a process dependent on the required geometry. A silicon oxide or silicon nitride film is deposited and is then transferred onto a glass substrate by anodic bonding. Finally, all of the silicon is removed by an anisotropic etching solution, and a polymer may be cast to give better mechanical strength.

One of the major disadvantages of the techniques described up to now is the bonding step that is expensive and can introduce alignment errors as can be seen in figure 13(*a*). Several techniques have been developed to close channels without any substrate transfer. For example, a variant of the

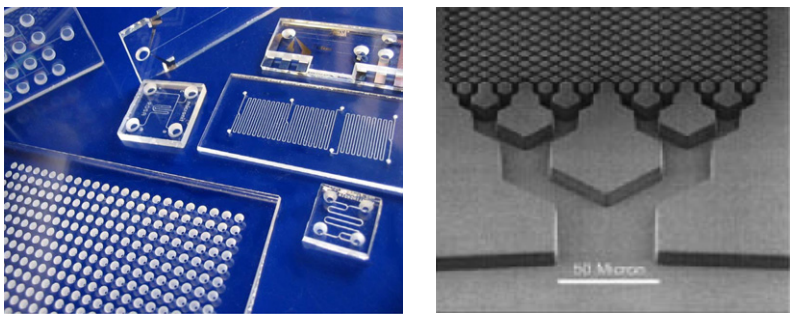
Table 1. Example combinations of structural and sacrificial layers [79].

Sacrificial layer	Structural layer	Release agent
Poly-Si	SiC, SiN	TMAH, KOH
Poly-Si	SiO_2	TMAH
Polyimide	Al, SiC, SiN, Ti	Plasma O_2
SiO_2	SiC, SiN, poly-Si, TiN	HF
SiO_2	Ti, Al	73% HF

SCREAM technology can be used to make closed structures with different profiles by sealing the structure with a film of polysilicon, silicon nitride or silicon oxide (figure 13(*b*)) [76]. With another technique, silicon is etched by wet etching through an array of small holes subsequently filled in by a silicon carbide film deposited by plasma enhanced chemical vapour deposition (PECVD), figure 13(*c*)) [77]. Buried channels can also be formed by alternating the porous silicon formation mode and the silicon electropolishing mode, in other words by varying the current during electrolysis of silicon in a hydrofluoric acid and ethanol mix [78].

2.1.2. Surface machining.

Sacrificial layer. Surface machining uses deposition or growth of thin layers on a substrate and selective etching of them. Unlike bulk machining, the structural material is the added thin layer and not the substrate. The use of a sacrificial layer illustrated in figure 14 is a fundamental concept in surface machining. In this example, the process begins by deposition of the sacrificial layer (*a*), conventionally a silicon oxide deposited by low-pressure chemical vapour deposition (LPCVD). After photolithography of this first layer, the structural layer is deposited, typically consisting of LPCVD polycrystalline silicon, and the accesses are defined (*b*). Finally, the sacrificial layer is etched and the structure is released (*c*). This technology can be used to make thin suspended structures. Table 1 shows different pairs of structural and sacrificial layers [79]. Attention must be paid to selectivity and isotropy of the etching process. In many cases, the structural layer is made of polycrystalline silicon and the sacrificial layer is made of silicon oxide, as in the multi user MEMS process (MUMPS) or in the SUMMiT V (Sandia Ultraplanar Multilevel MEMS Technology) process developed by Sandia National Laboratories and marketed by



(*a*) (*b*)

Figure 11. (*a*) Commercial glass chips [Micronit] [63] used with permission; (*b*) column of a 10 μm thick liquid phase chromatography monolith made by RIE of quartz [Purdue University] [70], reprinted with permission, © 1998 American Chemical Society.

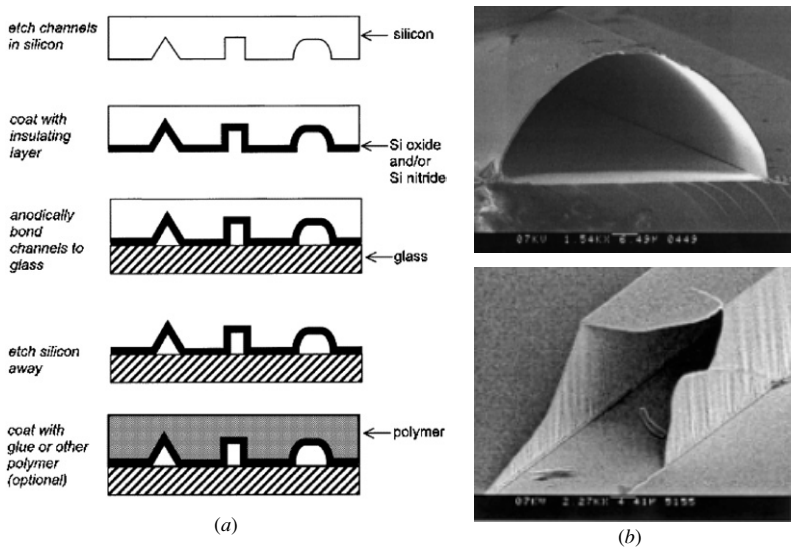


Figure 12. (*a*) μTIC manufacturing process [33] (© 2003 IEEE); (*b*) examples of μTIC [MESA+] [66] reproduced with permission, [75] © 1998, with permission from Elsevier.

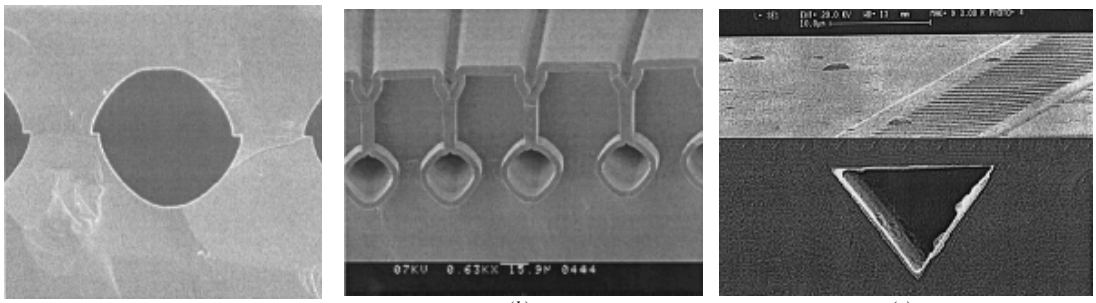


Figure 13. (*a*) Alignment error when bonding two substrates [MESA+], (*b*) channels made using a variant of the SCREAM technology [MESA+] [76] (© 2000 IEEE); (*c*) microstructure closed by a silicon carbide film [DaYeh University] [77], © 1999, with permission from Elsevier.

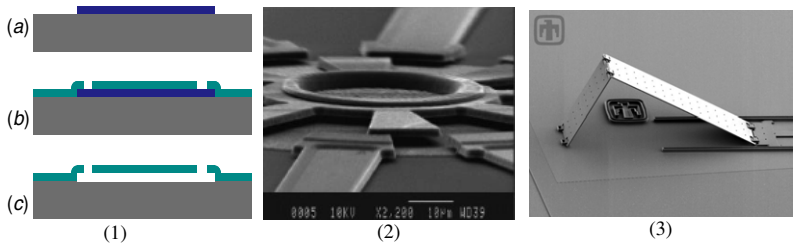


Figure 14. (1) Concept of sacrificial layer; (2) micromotor made by surface machining [LAAS] [82]; (3) micromirror made using the SUMMiT V technology [Sandia] [81].

the MEMX company in which five structural layers are stacked (figure 14(3)) [80, 81]. Another advantage of this technique is that it does not require a bonding step between substrates. However, the size of the structure is limited by the mechanical stress generated during the different technological steps.

SOI micromachining. One technique involving bulk machining and surface machining consists of using SOI (silicon-on-insulator) type substrates with a buried oxide as shown in figure 15(1). In this case, the first step consists of deep etching of silicon (*b*), followed by etching of buried silicon oxide by hydrofluoric acid to release the structure (*c*). It is easy to make monocrystalline silicon structures with this process that is commercially available from the TRONIC'S Microsystems company [83, 84].

Examples of microfluidic systems obtained by using glass or silicon surface micromachining. The MUMPS process was used successfully for manufacturing of silicon and glass microchannels, a layer of polycrystalline silicon being used as protection for etching in the latter case (figure 16(*b*)) [86]. However, there are several limitations. Firstly, due to the stress generated during the deposition process, the thickness of the sacrificial layers remains less than a few microns,

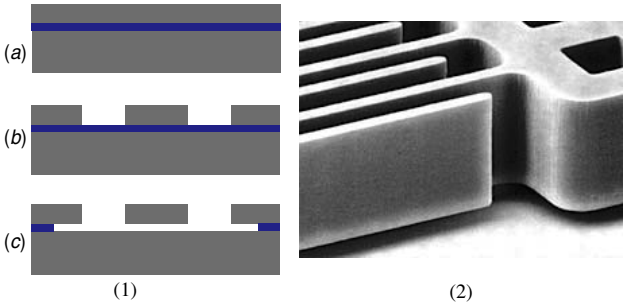


Figure 15. (1) Bulk micromachining and SOI; (2) 20 μm high comb made using the SOI technology [Draper] [85].

which limits the section of the channel. Furthermore, the maximum possible length using these techniques is small. As shown in figure 16(*a*), residues developed during etching of the sacrificial layer will agglomerate at the interface. Since the only way for the etching solution to regenerate itself in active species is diffusion, the etching rate progressively reduces with time. This can result in very long etching times and therefore requires excellent selectivity. This behaviour is illustrated in curve (*c*) in figure 16. It can also be seen that as the dimensions become smaller, the rate becomes slower.

One way of accelerating etching is to add accesses along the channel. These openings do not necessarily have to be closed afterwards to contain the flow during use. Under convenient flow conditions (pressure, surface tension, access height), the effects of surface tension can keep the liquid in the microchannel [86]. Otherwise, accesses can be sealed by the deposition, for example, of a LPCVD silicon carbide as described previously, or a PECVD silicon oxide, or even by evaporation of gold [77, 87, 88]. Another solution is to use materials that can be etched very quickly such as porous silicon, for which the etching rate may be as high as 10 mm min.$^{-1}$ [89]. However, its integration may be problematic and the surface roughness of the porous sacrificial layer will be transferred into the structural layer.

Although this technology cannot be used to make long microchannels, the weak thickness of the sacrificial films is an advantage for manufacturing nanofluidic systems. The realization of nanochannels by surface machining has also been demonstrated [91]. Electron beam lithography (EBL) is frequently used to achieve nanometric lateral dimensions, either directly or through a nanoimprint technique [87, 92]. Other technologies using conventional lithography have been used as shown in figure 17(*a*). For example, Tas *et al* have demonstrated a technique for transforming the thickness of a film into a channel width, using a deposition step followed by an RIE, and a process making use of sticking effects due to capillarity when a suspended structure is released by a wet etching method [93]. It should be noted that the etching time of the sacrificial layer is 15 h in the first case in a solution of KOH at 75 °C for a 0.64 mm long channel with a 40 × 90 nm section. The silicon nitride/polycrystalline silicon couple is used and has excellent selectivity. Lee *et al* have also used a technique in which the thickness of a silicon oxide becomes the lateral dimension of a microchannel [88]. The bottom of the structure is made of silicon nitride and the walls are made of amorphous silicon. The open structure is sealed after etching by deposition of a gold or silicon oxide film, which avoids problems related to excessively long under-etching times. However, these latter three methods are geometrically limited, for example for an intersection. Electrochemical etching of a sacrificial layer of copper has been demonstrated for nanochannels made of silicon oxide [94]. 4 min is sufficient to release the structure for a 100 nm by 300 μm section with a length of 2 cm.

Thicker sacrificial layers have also been used. Channels have been made using a thin silicon layer in an SOI wafer encapsulated by a diamond film as can be seen in figure 17(*b*) [96]. Etching times were then tested in trapezoidal channels for which the large base measured 73 μm, the small base 13 μm and the height 46 μm. 100 h were necessary to etch 2 cm in an HF/HNO_3 mix at 80 °C, and 350 h were necessary to etch 3 cm. Metals obtained by electrolysis can also be used as thick sacrificial layers. Figure 17(*c*) shows a detail of a microfluidic

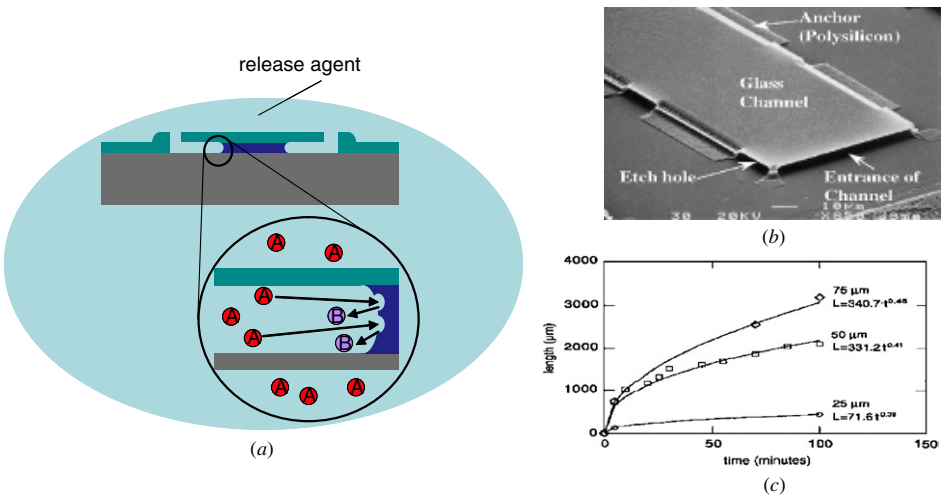
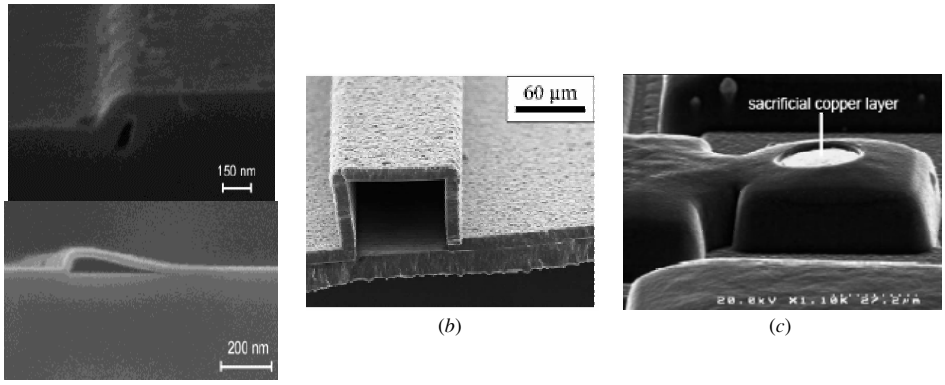
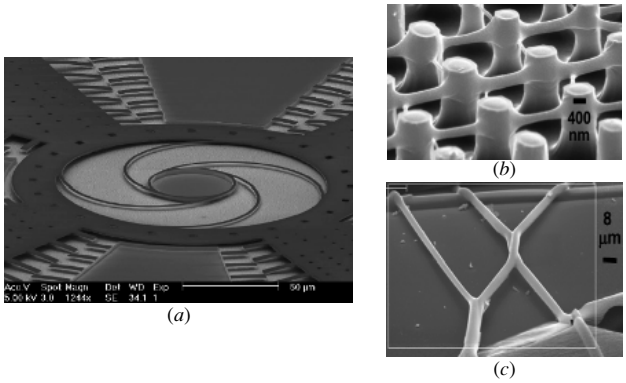


Figure 16. (*a*) Saturation at the interface during chemical etching; (*b*) microchannels made by MUMPS [Berkeley] [86], © 2004, with permission from Elsevier; (*c*) model (lines) and experimental data (symbols) for progressive release of a 100 μm wide and 25, 50 and 75 μm [90] deep microchannel (© 1997 IEEE).



(*a*) (*b*) (*c*)

Figure 17. (*a*) Examples of nanochannels made by surface machining [MESA+] [93], reprinted with permission, © 2002 American Chemical Society; (*b*) diamond microchannel made by combining SOI and surface machining [Uppsala University] [96], © 2001, with permission from Elsevier; (*c*) nozzle of a diamond ejector before etching of the copper sacrificial layer [Ulm University] [95], © 2005, with permission from Elsevier.



(*a*) (*b*) (*c*)

Figure 18. (*a*) Silicon carbide structure made using a polyamide sacrificial layer [Delft University] [79]; (*b*) PMMA wires pulled between two pillars by an AFM tip and (*c*) covered by glass after dissolution of the PMMA [Louisville University] [98], reprinted with permission, © 2004 American Chemical Society.

system also made of diamond [97]. The metal used is copper and the channels were up to 40 μm deep. 24 h were necessary to release the entire structure, but dimensions are not given.

Polymers can also be used as a sacrificial layer. They may be deposited simply using spin coating at low temperature, and some are photosensitive. For example, a 2–4 μm thick layer of polyimide was used to make the structure shown in figure 18(*a*) [79]. Polyimide is chemically resistant and withstands temperatures of up to 400 °C. However, baking temperatures are relatively high at more than 200 °C, and the layer is structured by an oxygen plasma through an aluminium mask. Complex micro- and nanometric structures have also been made by direct writing and pulling PMMA (polymethylmethacrylate) wires between two reservoirs with the tip of an atomic force microscope (AFM) [98]. The structures were then covered by a film of borosilicate glass (or other) deposited by sputtering, and the PMMA was then dissolved. Dissolution conditions were not given. The result is shown in figures 18(*b*) and (*c*).

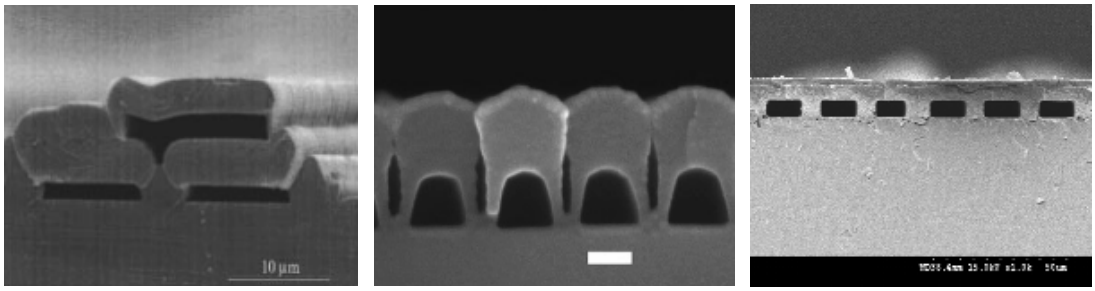
The problem of the dissolution time is still present but polymers can provide an innovative solution. Some polymers decompose thermally at a relatively low temperature without leaving any residue. Therefore, a sacrificial layer composed of such a material will decompose uniformly along the entire length of the channel, preventing diffusion problems related to chemical etching, with decomposition gases passing through the upper layer to prevent excessive overpressure. Different polycarbonates and polynorbonenes were used and covered by silica or silicon nitride films [99, 100]. Polycarbonates decompose at between 200 and 300 °C and polynorbonene decomposes at between 400 and 450 °C. The decomposition takes several hours. The thickness of films deposited by spin coating is not more than 15 μm and they are structured by RIE. The example of a three-dimensional structure made in this way is shown in figure 19(*a*). Nanochannels were also made using this technology, by direct electron beam lithography of a polycarbonate or by nanoimprint lithography [101, 102]. These polymers have also been made photosensitive so as to simplify the process [103–105]. The decomposition of the modified polycarbonate is within the 100–180 °C range. Silica channels covered by polyimide are shown in figure 19 [106].

One of the limitations of these processes is still the limited thickness of the polymer films. Deep dry etching of polymers is not as well controlled as etching of silicon and photoresists described in this section cannot be used to obtain layers thicker than 20 μm. However, the SU-8 photoresist, which can be used in very thick layers, can meet this challenge.

2.2. *Polymer technologies*

2.2.1. General about organic polymers. A number of different, polymer-based materials have been utilized for micro-manufacturing of various devices. In general, polymers are long chains obtained during a polymerization process that can be either in an amorphous or a partially crystalline arrangement.

The behaviour of polymers is controlled principally by the type of bonds existing between the chains [107, 108]. Thermoplastics, such as PMMA or polycarbonate (PC), are composed of chains that are not or only weakly bonded together. These polymers soften upon heating and harden when they are cooled down. Typically, thermoplastic materials are soluble in organic solvents. On the other hand, thermosetting polymers are cross-linked, not soluble in organic



(*a*) (*b*) (*c*)

Figure 19. (*a*) Three-dimensional structures [GeorgiaTech] [100] (© 2001 IEEE), (*b*) nanometric structures made by decomposition of polynorbonene (the white mark represents 100 nm) [Princeton] [101] and (*c*) micro channels made of silicon covered by polyimide obtained by degradation of a photosensitive polycarbonate at 170 °C [GeorgiaTech] [106].

Table 2. Properties of polymers compared with properties of silicon and glass [112–118].

Name		T_g (°C)	T_m (°C)	Thermal expansion coefficient (ppm K^{-1})	Structure
COC TOPAS 5013	Cycloolefin copolymer	140	/	nd	Amorphous
PMMA	Polymethylmethacrylate	105	/	70–77	Amorphous
PC	Polycarbonate	150	/	66–70	Amorphous
PS	Polystyrene	100	/	30–210	Amorphous
POM	Polyoxymethylene	−15	160	80–120	Semi-crystalline
PFA	Perfluoralkoxy copolymer	nd	310	nd	Semi-crystalline
PVC	Polyvinylchloride	90	/	50–180	Amorphous
PP	Polypropylene	−20	170	100–180	Semi-crystalline
PET	Polyethylene terephtalate	80	265	20–80	Semi-crystalline
PEEK	Polyetheretherketone	150	340	50–110	Semi-crystalline
PA	Polyamide	50	260	80–95	Semi-crystalline
PVDF	Polyvinylidenefluoride	40	210	80–140	Semi-crystalline
PI	Polyimide	350	/	30–60	Amorphous
LCP Vectra A950	Liquid crystal polymer	/	280	0–30	Semi-crystalline
Crosslinked SU-8		200	/	50	Amorphous
Crosslinked PDMS Sylgard 184	Polydimethylsiloxane	−128	/	310	Amorphous
Borofloat glass		525	/	3.25	Amorphous
Silicon		/	1414	2.5	Crystalline

solvents and they must be shaped before cross-linking. For example, epoxy resists and rubber after vulcanization represent such materials.

Polymers are commonly characterized by the vitreous transition temperature (T_g), the melting temperature (T_m) and the decomposition temperature. Table 2 lists T_g, T_m, thermal expansion coefficients and the structure of polymers commonly employed for microfabrication of devices in comparison with monocrystalline silicon and amorphous glass. These values are given for guidance and may vary from one supplier to another. For more information on the polymer-based materials, the reader should consult well-established text books, such as Ward [109] or Young and Lovell [110]. There is a wide choice of different polymer-based materials and it is easy to understand the interest in integrating these materials in micro-manufacturing. For example, polyetheretherketone (PEEK) resists temperatures of 250 °C and is very resistant chemically, while thermoplastics, such as cycloolefin copolymer (COC), PMMA or PC are optically transparent. These materials are electrical insulators but can be filled with appropriate powders to make them conducting or to change their magnetic properties. Many are biocompatible and some, like polycaprolactone (PCL), are biodegradable [111]. The price of a material like PMMA is 10–100 times lower than the price of glass [107]. There are already many collective manufacturing processes. Their application of the polymers in the microsystems field is described in the following sections.

2.2.2. Replication techniques. Heckele *et al* published an excellent review of the thermoplastics micro-moulding in 2004 [113]. One of the first examples of micro-moulding was published in 1970 by a Group from Princeton, the application being a system for making holographic recordings on tape for television [119]. In this study, the micrometric patterns were reproduced by rolling a nickel matrix onto a vinyl tape. The first applications were published in the middle of the 1990s. They made use of techniques adapted from well-known plastic replication technologies [120]. The diagram in figure 20 summarizes the different steps involved in the manufacturing of micro-fluidic elements by replication.

Manufacturing of the master. The technological process begins with manufacturing of the master that will be used as a negative for the actual replication. This master must satisfy several conditions. Firstly, it must have a low roughness, typically less than 100 nm RMS (root mean square), to facilitate mould removal [107]. A slight inclination is even preferable if compatible with the application

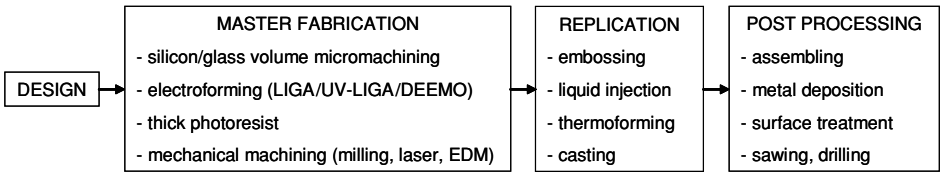


Figure 20. Steps involved in a replication.

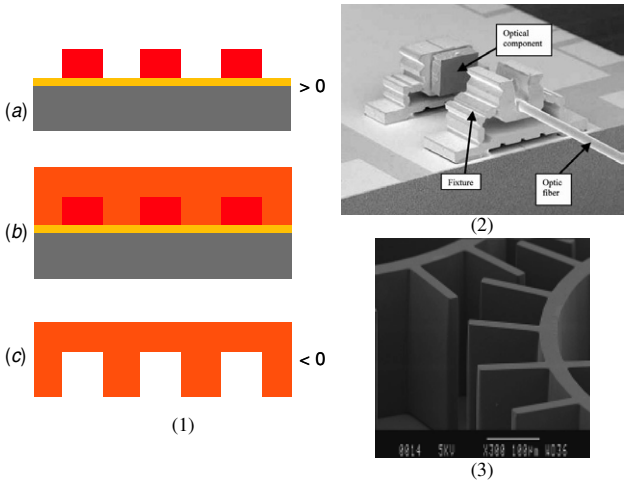


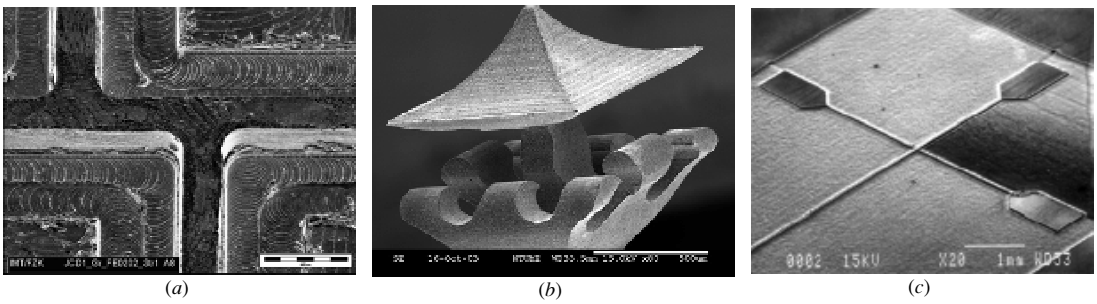
Figure 21. (1) Creation of a metal mould by electrolytic deposition from a fragile structure; (2) optical coupling elements made by LIGA [AXSUN] [124]; (3) SU-8 micro-gear [LAAS].

[113]. Furthermore, the material used must be sufficiently hard and ductile to resist several moulding cycles. In fact, the master material will depend on the process, the type of plastic and the quantity of parts to be provided. Several techniques were developed at Forschungszentrum Karlsruhe (FzK), a pioneer research centre in the field, one of the first and best known techniques being LIGA, the German acronym for Galvanoformung und Abformung, namely lithography, electroforming and moulding [121]. The master manufacturing technique with the LIGA technique is summarized in figure 21(1). The process begins by (*a*) the deposition of a thin metal layer (that will be used as an electrode during the electrolytic deposition), then x-ray lithography of a thick layer of PMMA. Since x-rays are weakly absorbed, very thick structures can be obtained (several tens of micrometres) with excellent aspect ratios. However, although the resolution is excellent, this lithography is very expensive as it requires the use of a synchrotron radiation. After the PMMA has been structured, the electrolytic growth (*b*) of the metal is performed, followed, after polishing, by (*c*) the dissolution of the resist that releases the master. Nickel or nickel-based alloys (NiCO, NiFe) are frequently used.

Techniques for three-dimensional machining of silicon have also been used to make masters. The trapezoidal shape obtained by anisotropic wet etching facilitates mould removal and the process can achieve a very low roughness but the geometry is limited, for example in the case of an intersection [122]. The DRIE process can be used to obtain straight structures with an excellent aspect ratio but roughness will be higher for higher etching rates. Since silicon is relatively fragile, it can be mounted on another support or it can be used as an intermediary, like the PMMA structure in the LIGA technique, to make a metal master. The DEEMO (deep etching, electroplated, moulding) process consists of etching silicon by DRIE, and then growth of a metal layer by electroforming before using it for replication [123].

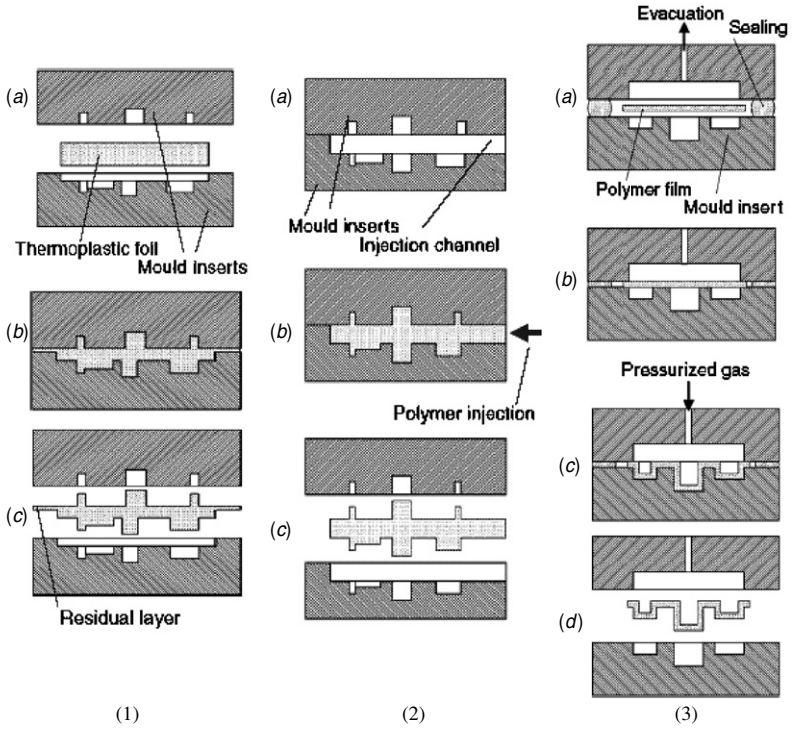
One means for avoiding the cost of x-lithography and the roughness of DRIE is to use conventional photoresists for electrolytic growth of the master. However, the UV absorption of most classical resists is too high, so that they cannot be used more than a few tens or even about a hundred microns thick [125, 126]. SU-8 is an epoxy negative photoresist produced by Shell Chemical and made photosensitive by the addition of triarylsulfonium salt [127]. It was developed by IBM at the beginning of the 1990s, and can be used to realize structures more than a millimetre thick with aspect ratios that were previously only possible by x-lithography, as illustrated in figure 21(3). Since the cost of the process was radically reduced by the use of a traditional aligner instead of a synchrotron, it is frequently called the 'poor-man's' LIGA, or the UV-LIGA. Products manufactured by the Swiss company MIMOTEC include plastic and metal clockwork components using this technology [128]. One of the encountered difficulties is that it is very difficult to remove SU-8 once it has been cross-linked. In the MIMOTEC process, it is removed using a high-pressure water jet. It can also be peeled without dissolving it using a solution of potassium hydroxide [129]. However, this disadvantage may be an advantage if SU-8 is used as a structural material, as will be demonstrated later. This photoresist can also be used on a silicon substrate as a master. However, although such a structure is suitable for prototyping, it cannot resist more than a few tens of thermal cycles, while metal components can resist thousands. This is due to the difference (factor of ten) between the thermal expansion coefficients of SU-8 and silicon [130].

Conventional milling techniques can also be used for resolutions of the order of 50–100 μm. Figure 22 shows an example of a master obtained with CNC (Computer Numerical Control) tools (*a*). The roughness on the walls is of the order of 200 nm. If the least rough structures are obtained with diamond cutters, the smallest possible diameter for this type of tool is 200 μm which limits usage [113]. Steel tools may be used for smaller dimensions, but at the detriment of the surface condition. Micro electrodischarge machining (μEDM) is another process that has appeared in the microtechnologies field [131]. In this case, metal is evaporated by very local heating by applying an electrodischarge between the material to be machined and the tool. Complex shapes can be obtained but the surface condition is not as good as with high speed machining. Finally, laser machining appears to be a very



(*a*) (*b*) (*c*)

Figure 22. (*a*) Details of a master machined by milling [FzK] [134], © 2004, with permission from Elsevier, (*b*) micropagoda made by μEDM [Taiwan University] [131]; (*c*) master made by laser machining with different roughnesses [113].



(1) (2) (3)

Figure 23. (1) Embossing, (2) liquid injection and (3) thermoforming [113].

promising option in terms of resolution and the material that can be treated [132, 133]. Another advantage of these techniques is a good control over the angle of the walls, which is impossible or difficult with the other mentioned means.

Replication. The replication technique most frequently used in microtechnology is hot embossing. As shown in figure 23(1), a thermoplastic sheet is heated to above its T_g and it is pressed under a vacuum. The assembly is cooled and the formed part is separated from the mould.

On the other hand, the best known and most frequently used technique under industrial conditions is injection moulding shown in figure 23(2). The so-called variotherm process includes the following steps. Firstly, the tool including the microstructured master is closed, heated to above the T_g of the polymer and put under vacuum. The thermoplastic is then heated and injected and the formed part is cooled and finally stripped from the mould. The fact that the polymer is continuously supplied above its T_g makes the cycle for this process much shorter than the embossing cycle, which explains why it is used in almost all series production for macroscopic formats [107, 113]. However, embossing machines are much more simpler, which makes them more suitable for small and medium series and for research institutes. Furthermore, the distance to be travelled by the polymer is longer for liquid injection, which imposes higher temperatures and therefore a higher thermal stress during the cooling step. Therefore, the replication quality is often better for embossing, which makes it much more suitable for example for optical microstructures. Several companies such as Jenoptik, EVG and Battenfeld market machines dedicated to these applications [135–137].

A combination of these two techniques (injection compression moulding) is used for manufacturing CDs or DVDs. In this case, the polymer is injected hot into the mould that is still open and is then pressed. This solves the problem of injection into a small volume. Furthermore, the thermoplastic does not need to be heated by the tool as

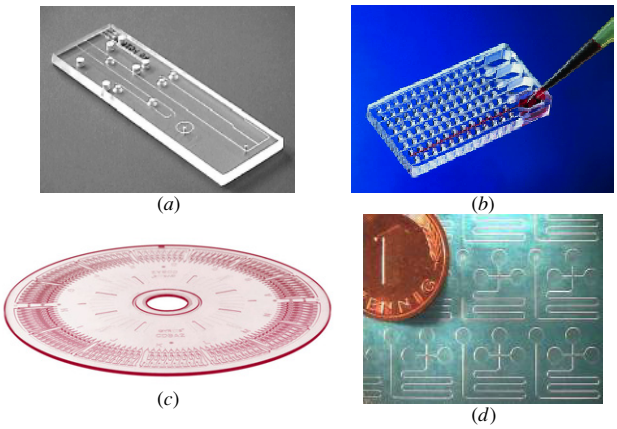


Figure 24. (*a*) Microfluidic circuit made by embossing of PMMA [ISAS] [140], © 2002, with permission from Elsevier; (*b*) microwell plate made by injection molding of PS [Boehringer Ingelheim microParts] [155] used with permission; (*c*) CD format lab-on-chip made by liquid injection [Gyros] [156], used with permission, and (*d*) CE system obtained by thermoforming [FzK] [157].

in traditional embossing, which can reduce cycle times. This technique is particularly well adapted for thin parts.

Another variant of injection called reaction injection moulding can be used to make elastomer and thermosetting parts. Two components, the polymer and the cross-linking agent, are then injected into the mould. The technique was initially used at FzK in the middle of the 1980s and was abandoned due to problems related to mixing components. Reaction times were also relatively long. However, the use of photoinitiators instead of thermal initiators can now considerably reduce cycle times.

Finally, another technique adapted to replication at the macroscopic scale is thermoforming shown in figure 23(3). A thermoplastic film is inserted into the tool, put under vacuum, clamped and the temperature is increased. A pressurized gas then brings the film into contact with the patterns, and the part is cooled before the mould is removed. Thermoforming cannot be used to obtain large aspect ratios because the polymer cannot be heated too much to prevent it from becoming too permeable when the gas blows it into contact with the master.

Realizations. One of the first examples of polymer components for microfluidics dates from 1997 [122]. The microchannels were formed by embossing of a metal wire or a silicon mould in a piece of PMMA. In the case of the silicon master, the plastic/PMMA assembly was simply clamped between two aluminium plates and left for 10 min in an oven at 105 °C. Printing quality was poor at lower temperatures, and bubbles formed at higher temperatures. After holes had been drilled, the microchannels were sealed by clamping another piece of PMMA in contact with the first one, also between two aluminium plates and the assembly was left for 10 min at 108 °C. Microchannels were thus made with a section 43 μm by 33 μm. This example is representative of the simplicity of the process once the master has been manufactured. The same group has demonstrated manufacturing of microstructures by embossing in PMMA and polyester at ambient temperature and high pressures [138].

Madou *et al* presented microfluidic structures made in a compact disk format using CNC tools directly or by embossing in PC with a master obtained by electrolytic growth of nickel on SU-8 structures [139]. The same type of process is used by Grass *et al*, also with the integration of platinum electrodes structured by a lift-off process, which prevents etching of the metal and therefore the use of aggressive chemicals [140]. The result is shown in figure 24(*a*). The use of wet-etched glass masters has also been demonstrated [141]. Polymer masters have also been used, to facilitate manufacturing and to extend their life. In [142], structures with a minimum dimension of 40 μm were made by embossing a PDMS stamp in PMMA. Similarly, Koerner *et al* use a fully epoxy resist master and reproduce patterns with a resolution of 3 μm [143]. A modification of the surface of PMMA is also presented to improve sealing.

Several alternative bonding solutions between substrates have been proposed to limit induced deformation or other undesirable effects, use of adhesive, solvent-assisted bonding, vacuum-assisted bonding, microwave-assisted bonding, or bonding under a bath of boiling water [144–148]. In the article by Kelly *et al*, a wax is introduced before solvent-assisted bonding to avoid deformations, and is then removed at the end of the process by melting it [149]. Other materials such as COC with excellent transparency and that are much more chemically resistant than PMMA or PC, have also been used [150, 151]. Although embossing has been extensively described in the literature, few groups have reported solutions based on liquid injection due to the relative complexity of the equipment. Nickel masters have been manufactured by DEEMO for replication by injection of acrylic parts closed by laminating a mylar thermoadhesive film [152]. In the work by Dang *et al*, the nickel mould is manufactured by a process similar to LIGA and injected parts are closed by laminating a pressure-sensitive adhesive [153].

Although injection is not frequent in research institutes, many microfluidic products are made using this process by companies such as Microfluidic ChipShop, Boehringer Ingelheim MicroParts (formerly Steag MicroParts) or Gyros [154–156]. Several examples are shown in figure 24. Finally, microfluidic systems were also made by thermoforming of a polystyrene (PS) film, and gold electrodes have been deposited on these structures through an opening before forming ('shadow-mask' technique) [157].

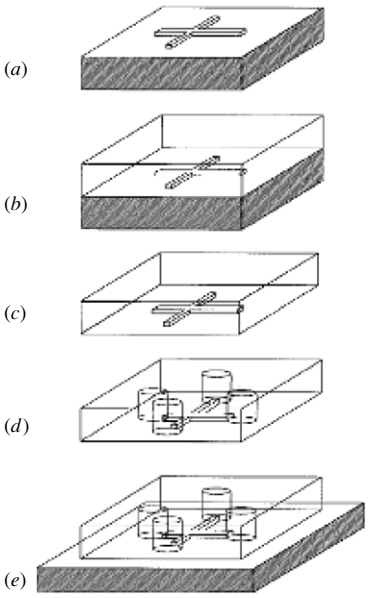
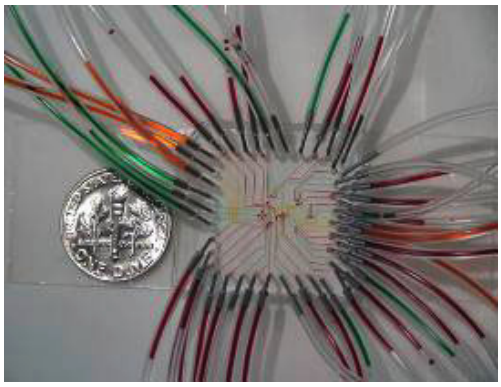


Figure 25. The PDMS casting process [4], reprinted with permission, © 1997 American Chemical Society.

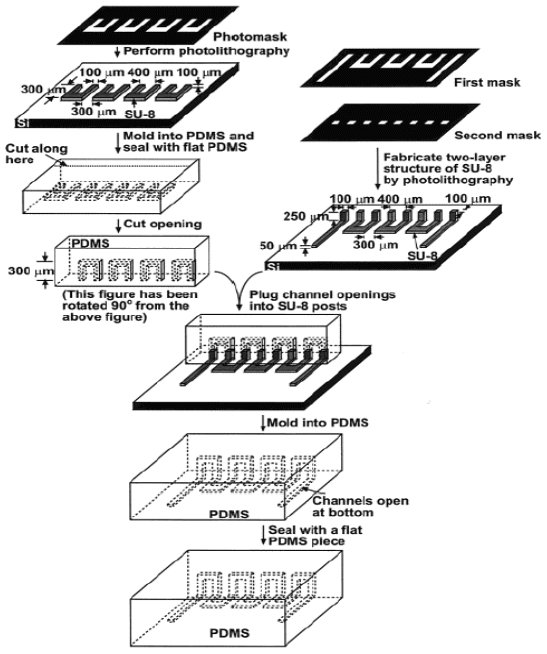
Casting. However, the most frequently used replication technique in microfluidics at the moment is undoubtedly casting of PDMS (figure 25) [4, 5]. The process begins by manufacturing of the mould, frequently by bulk machining of silicon or by thick SU-8 photolithography (*a*). A mixture of silicone rubber and cross linking agent is cast on this matrix (*b*). After cross linking, the elastomer is simply peeled off the substrate (*c*), accesses are created, frequently with a part holder (*d*), and the part is transferred onto another piece of PDMS (*e*). This moulding method was initially used by the G M Whithesites team in Harvard for advanced lithography techniques such as 'microcontact printing' (μCP), 'micromoulding in capillaries' (MIMIC) and by IBM for 'microfluidic networks' (μFN) [158–160].

The first advantage of this technology is its ease of implementation since there is no need for tooling or for development of the system (temperature, pressure). The mould is damaged less quickly than in the techniques described previously because the thermal cycle is usually lower and stripping is easy since PDMS is an elastomer. This flexibility also enables creation of a very conform contact to another substrate, for example a silicon or glass plate or another piece of PDMS. This property makes it very easy to close PDMS systems because all that is necessary is to place the open structure on a sufficiently flat substrate so that a reversible assembly is created due to Van der Waals type molecular interactions. Si–O–Si bonds are created and this assembly becomes irreversible after oxidation of the two parts, for example by treatment with oxygen plasma. Finally, PDMS is biocompatible, has excellent transparency and the replication quality is excellent down to nanometric scales. All these advantages mean that PDMS is a preferred material for laboratories that do not have advanced technological tools.

A complex system made in this way is shown in figure 26(*a*). Three-dimensional systems were built level by level by stacking the layers [162]. The alignment was made under a microscope assisted by guides structured in PDMS with an optimum resolution of the order of 15 μm. Wu *et al* present another technique for manufacturing three-dimensional microfluidic networks shown in figure 26(*b*)



(*a*)



(*b*)

Figure 26. (*a*) PDMS microfluidic chip and its connections [UCLA] [101]; (*b*) manufacturing process for a 3D network made of PDMS [Harvard] [163], reprinted with permission, © 2003 American Chemical Society.

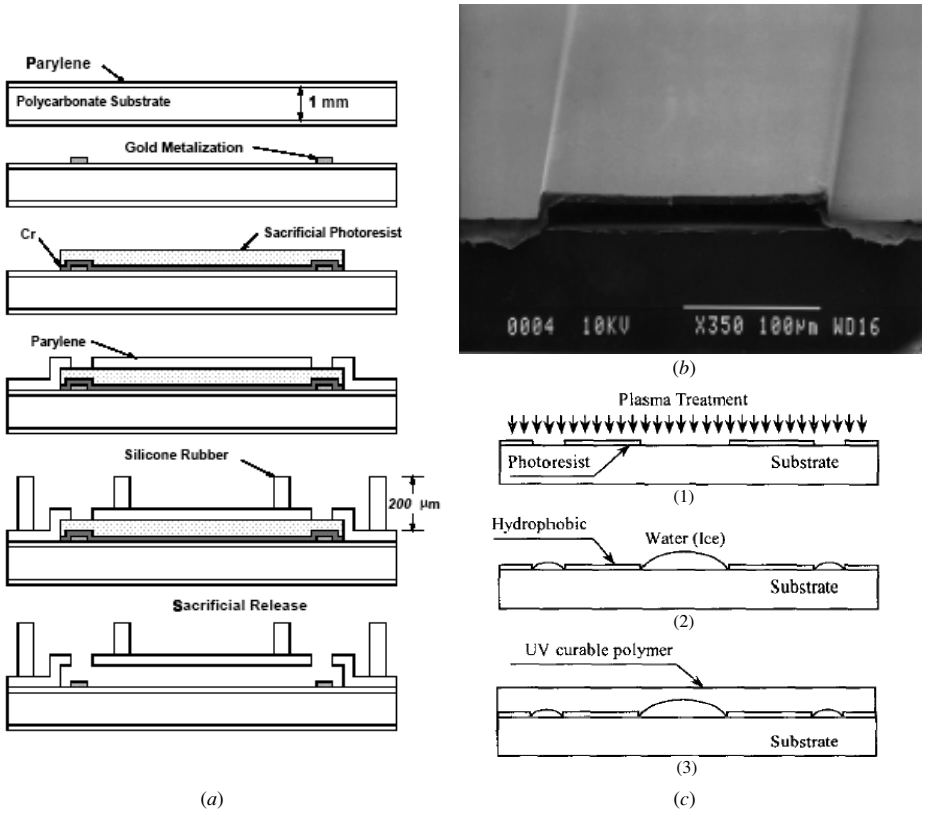


Figure 27. (*a*) Manufacturing process for a parylene system; (*b*) example [Michigan University] [169]; (*c*) frozen water as the sacrificial layer [Berkeley] [171] (© 2003 IEEE).

[163]. In this process, a first PDMS chip is cut out and turned at an angle of 90°, connected on a second mould before a second casting is done. These two examples are characteristics of the difficulties encountered in making three-dimensional networks, and more generally assembly of microfluidic structures made by replication. Another disadvantage is due to the material itself because PDMS swells in most solvents [164].

To overcome these drawbacks, a photosensitive fluoropolymer with excellent chemical resistance has been evaluated [165]. In the article by Muck *et al*, a photosensitive PMMA was cast on silicon structures obtained by anisotropic wet etching [166]. The casting process was also used for replication of various epoxy resists [167]. These resists usually have good transparency, weak auto-fluorescence and good chemical resistance. Sudarsan *et al* use an elastomer thermoplastic gel to make a reconfigurable microfluidic network [168].

2.2.3. Direct structuration techniques and SU-8 technologies. Silicon surface machining techniques have also been adapted for use by polymer technologies, again with the objective of avoiding the expensive assembly step and to facilitate integration.

One of the first examples of this type of microfluidic integration is described in figure 27(*a*) [169]. The structural material was parylene, an inert, transparent, biocompatible material that has the advantage that it is deposited in the vapour phase at ambient temperature, resulting in a very homogenous and low stress film [170]. The process begins by deposition of parylene on a polycarbonate substrate so as to protect it during subsequent chemical treatments. The electrodes are created by evaporation and photolithography of a gold film. A chromium layer and then the sacrificial resist are deposited. The chromium layer improves the resolution of photolithography of the resist. After the resist has been structured, the uncovered chromium area is etched and a second layer of parylene is then deposited. Accesses are opened with an oxygen plasma then a photosensitive silicone rubber is used to create reservoirs and finally the sacrificial layer is dissolved (36 h for 1.5 cm) and the residual chromium is etched. The section of a 20 μm high and 200 μm wide microchannel is shown in figure 27. This process demonstrates possibilities made available by low temperature depositions. The introduction of polymers as construction materials, for example deposited in the vapour phase as parylene or simply with a spin coater, allows the use of sacrificial layers that would not have resisted deposition conditions used in conventional silicon technology. One striking example illustrating this concept is the use of frozen water as the sacrificial layer demonstrated at Berkeley and illustrated in figure 27(*c*) [171].

SU-8 has many qualities that make it an ideal structural material for microfluidics. It is transparent, has a high chemical resistance and is biocompatible [172]. It is also easy to deposit with a spin coater in a thickness range varying from one micron to one millimetre, can be worked at less than 100 °C and can be used to make structures with excellent aspect ratios. The challenge is to find sufficiently thick sacrificial layers so

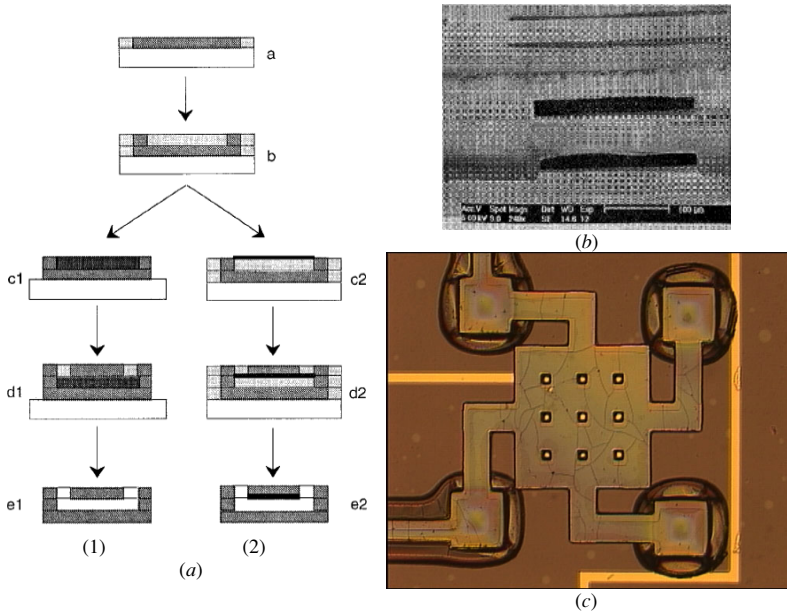


Figure 28. (1) Manufacturing process for microfluidic structures made entirely of SU-8 by (*a*) filling and (*b*) use of a metal mask; (2) three-dimensional network made using this process [EPFL] [90] (© 1997 IEEE); (3) micro-mirror made entirely of SU-8 with an SU-8 sacrificial layer [LAAS] [174].

as to keep the geometric advantages provided by the use of SU-8. Two strategies were developed in [90]. The first idea described in figure 28(*a*, 1) was to fill the open structure made of SU-8 with different materials (thermoplastics, waxes, epoxy resists) before depositing the final layer, opening accesses and removing the sacrificial material. The second technique (figure 28(*b*, 1)) used the non-cross-linked SU-8 resist itself, as the sacrificial layer. Since SU-8 is a negative resist, a metal mask had to be used to prevent cross-linking of the sacrificial level. Relatively long etching times were necessary for both processes, as can be seen in figure 16(*c*). Three-dimensional structures were obtained (figure 28(2)). The use of SU-8 as a sacrificial layer has recently been described for manufacturing of suspended metal structures [173]. The validity of a similar process for manufacturing micro-mirrors entirely of SU-8 was also demonstrated at LAAS-CNRS, leading to actuation voltages significantly lower than their silicon equivalents (figure 28(3)) [174].

Several groups describe techniques for partial exposition of an SU-8 layer so as to simplify the process. Examples of results are shown in figure 29. In the article by Tay *et al*, a proton source is used [175]. Exposition is done in two steps, a complete exposition that creates walls and a partial exposition for the cover, the dose being adapted depending on the situation. This process was also demonstrated in PMMA [176]. Furthermore, a UV laser was used by varying its focus [177]. Finally, this approach was demonstrated by Chuang *et al* with a conventional aligner using an anti-reflection coating so as to control the exposure depth [178].

Other types of sacrificial layers have also been used in association with polymer structural layers. The electrolytic copper of a printed circuit is used to make suspended structures of SU-8 [179]. Nanochannels made of polyimide, a material that also has a high chemical resistance, excellent mechanical and electrical properties but also strong autofluorescence, have also been made by etching a sacrificial layer of aluminium [180]. 20 h were necessary to etch 4 mm channels.

Thermodegradable sacrificial layers have also been used to prevent prolonged exposure to chemical products. Various dielectric materials such as benzocyclobutene (BCB), an Avatrel encapsulating product, SU-8 and polyimide were used as structural layers combined with sacrificial materials discussed above [99, 100, 105]. Jayachandran *et al* discuss the compatibility between structural and sacrificial materials, particularly at the level of solvents [106]. Incorporation of a silicon oxide layer deposited by PECVD is suggested to solve problems that arise.

Other techniques combine structuring of a polymer layer on a substrate, frequently made of silicon or glass, and bonding with another substrate. The UV laser ablation process for manufacturing microfluidic structures was reported as early as 1997 [181]. PS, PC, acetyl-cellulose and PET substrates have been structured in this manner and sealed by laminating a film of PE/PET at 135 °C. An example of a process used at MIT in 2001 is shown in figure 30 [182]. Two layers of SU-8 were deposited one after the other and structured on a silicon substrate, the first layer defining accesses and the second layer defining microchannels. A second pyrex substrate supporting an annealed but unexposed SU-8 layer is transferred onto the first substrate at 65 °C. Exposure takes place through the pyrex followed by the second annealing, and the silicon is removed. However, the efficiency of this type of transfer is low [183]. In the article by Lin *et al*, a 1 mm thick layer of SU-8 obtained by casting is glued between two glass substrates [184]. Complex structures were made using an inclined lithography process as can be seen in figure 30(*c*), closed for the test by a single piece of PDMS [185]. PMMA was used as the adhesive layer between SU-8 structures and a glass substrate [186]. A very thin layer of SU-8 can act as glue to prevent the occlusion in the microchannels [187, 188]. Substrates supporting SU-8 structures may be bonded

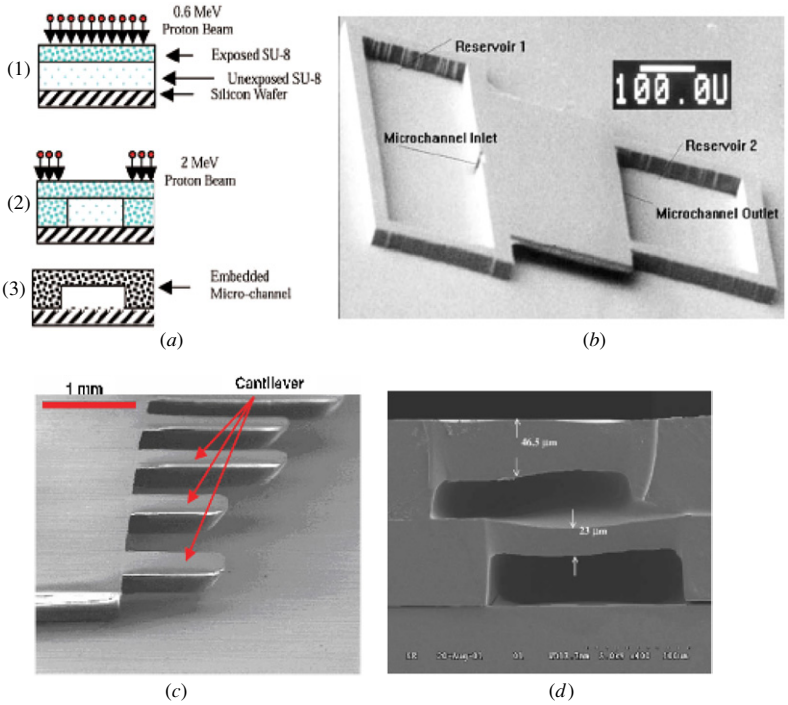


Figure 29. (1), (2) Partial exposition of an SU-8 layer by proton radiation [Singapore National University] [175], (3) partial exposition by a UV laser [Boston University] [177] and (4) by a conventional aligner associated with an anti-reflection coating [Tsing Hua National University] [178], © 2003, with permission from Elsevier.

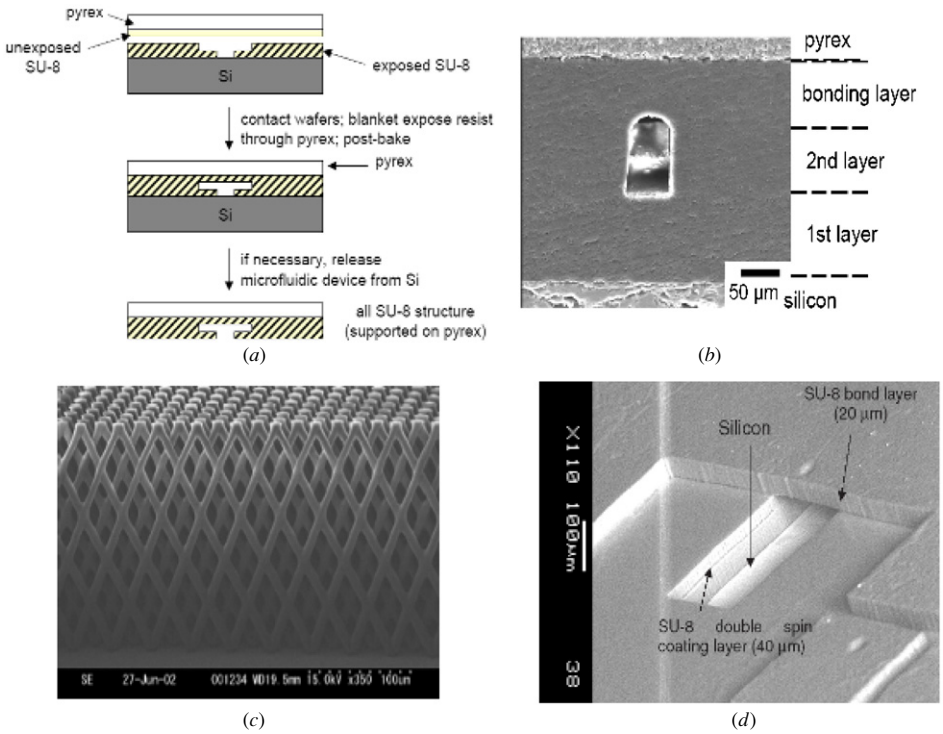


Figure 30. (*a*) Production of a microfluidic structure by photolithography of SU-8 and adhesive bonding and (*b*) example result [MIT] [182]; (*c*) filter made of SU-8 by inclined lithography [Waseda University] [185] (© 2003 IEEE); (*d*) three-dimensional structure after removal of the upper substrate [Ikerlan] [189].

by applying a pressure of 3 bars for 20 min at 100–120 °C in a dedicated equipment, also to prevent occlusion [189]. Three-dimensional networks may thus be created by successive transfers [190]. Improved homogeneity was demonstrated as a result of leaving non-cross-linked zones on the encapsulated parts between the structures [191]. Another way of improving

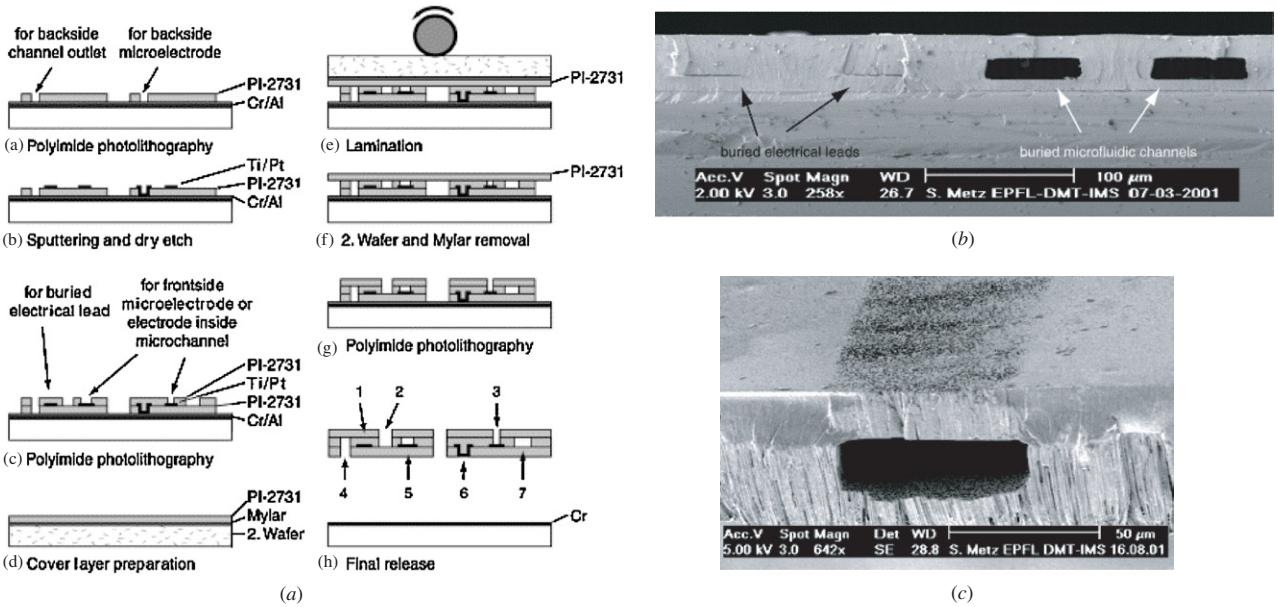


Figure 31. (*a*) Process for transfer of polyimide films by rolling [195] and (*b*) example results (© 2004, with permission from Elsevier); (*c*) integration of a filter [EPFL] [196].

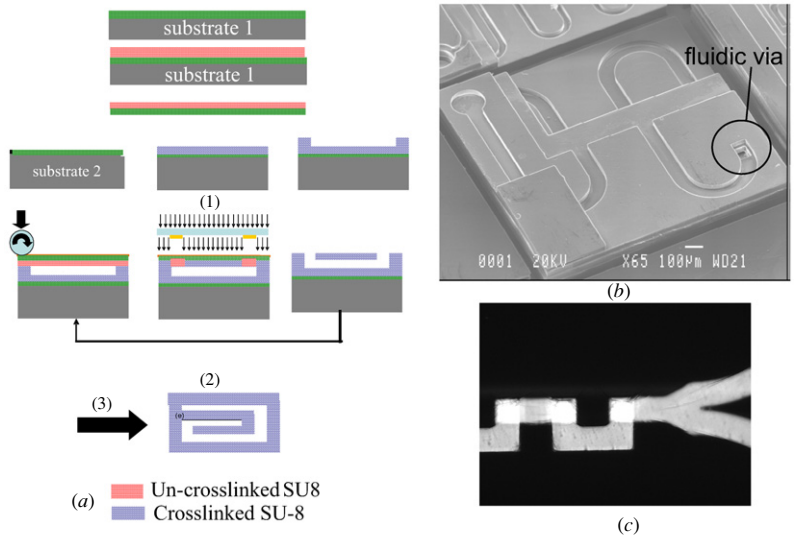


Figure 32. (*a*) Schematic description of the SU8 multilevel process, (*b*) section through a 3D microchannel made with 5 SU-8 levels, (*c*) injection of a fluorescent tracer in a 3D chaotic mixer with a 40 by 35 μm section [LAAS-CNRS] [200].

the homogeneity consisted of transferring a semi cross-linked film of SU-8 through a flexible film and not through a rigid substrate, so as to obtain a conform contact [192].

This idea also included the use of laminating photosensitive dry films. This technique is simple, inexpensive and avoids the imprecise alignment and assembly step in a wafer bonder. A Riston film marketed by Du Pont de Nemours was laminated on SU-8 and Riston structures [193]. The process described in figure 31 was developed to transfer polyimide films by lamination [194]. Integration of electrodes and a filter were also demonstrated [195, 196]. A film with properties similar to SU-8 but incapable of achieving such good aspect ratios was used [197].

An approach for manufacturing multi-level microchannel systems was reported [198]. As shown in figure 32(*a*), it consists of manufacturing films of SU-8 that are still photosensitive on a flexible support and stacking them before structuring. Unlike other previously proposed techniques, the use of a flexible support improves the homogeneity of bonding and facilitates its removal, since it can be simply peeled off after the transfer. This enables stacking of successive layers and manufacturing of the 3D network while maintaining the homogeneity of the surface of microchannels. Furthermore, the fact that the film is transferred before the photolithography step enables an excellent level-to-level alignment using only a conventional photolithography aligner. The process begins by manufacturing open microchannels made of SU-8 by conventional techniques (1). A film of SU-8 that is still photosensitive is manufactured and laminated on this microstructure (2) and then exposed to UV and developed. Another SU-8 film can be made and laminated in this step, so as to obtain a three-dimensional structure (3). Figure 32(*b*)

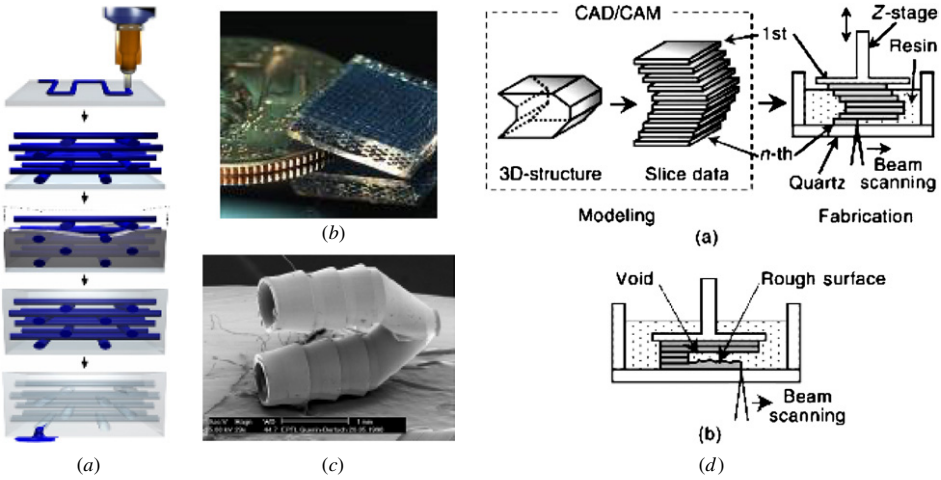


Figure 33. (*a*) Direct writing process using fugitive ink and casting in an epoxy resin and (*b*) photograph of a device [Illinois University] [202], reproduced with permission; (*c*) fluidic connector made by microstereolithography [EPFL] and (*d*) general principle of microstereolithograpy [203], © 2002, with permission from Elsevier.

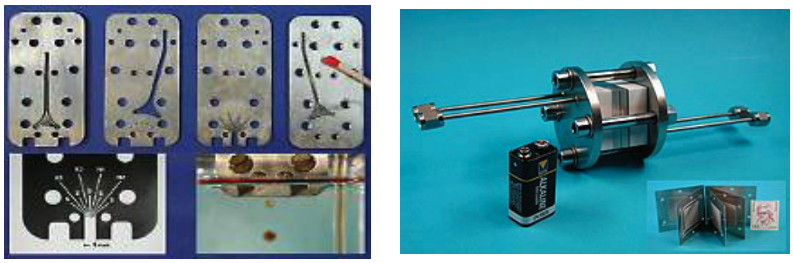


Figure 34. Metallic microreactors (*a*) mixer; (*b*) gaseous phase reactor [IMM].

shows the example of a microchannel running on several levels with a fluidic via, in which five levels of SU-8 are stacked. Figure 32(*c*) shows a sealing test by injection of a fluorescent tracer in a 3D chaotic mixer with microchannel section of 35 μm by 40 μm and length of several centimetres. Note that this technique was also tested successfully to make different suspended structures such as cantilevers or membranes, over lengths varying from 10 μm up to 1 mm. Strategies were developed to modify SU-8 surfaces, so as to have a generic technology for labs-on-chip [199, 200].

Finally, less conventional processes were also evaluated. For example, a wax was deposited level by level by a robot and then cast in an epoxy resist as can be seen in figures 33(*a*) and (*b*) [201, 202]. The stack is then heated and liquid wax is evacuated. A network of lines with dimensions between 10 and 500 μm can thus be created. Manufacturing of a demonstrator comprising 104 layers with 200 μm channels has been recently demonstrated. Microstereolithography is a prototyping technique used to create complex objects as shown in figure 33(*c*). This method was used to make an acrylic chip on an array of photodetectors in which a liquid photoresist is polymerized level by level [203].

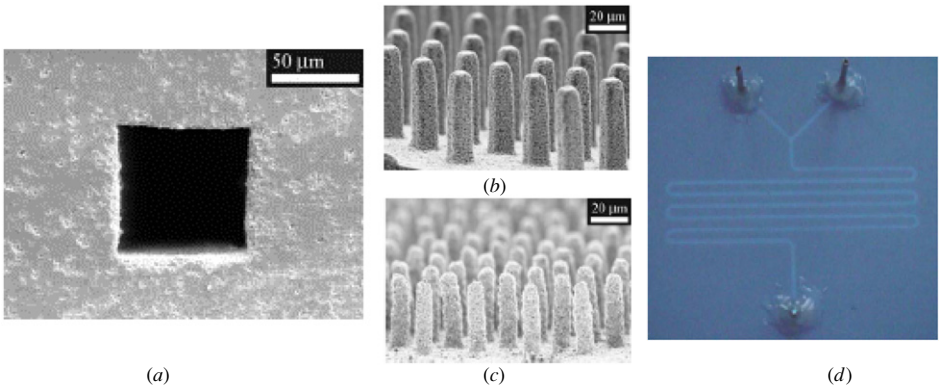
2.3. Other materials

Microfluidic elements made of metal or ceramic were also made, particularly for the microreaction field in which high temperatures and very aggressive chemicals can be used and in which good thermal conductivity is frequently required [204–207]. LIGA (if the moulding step is omitted), CNC machining techniques, laser machining and micro electro discharge machining associated with different bonding techniques are used for making these metallic structures, two examples of which are shown in figure 34. The dimensions of the microchannels are often larger than 100 μm.

Ceramic structures can be made by replication. The examples shown in figures 35(*a*)–(*c*) were made by imprinting in a PDMS mould before being sintered at 1280 °C [208]. For multi-level structures, the subassemblies are laminated when they are still in the green state. Mechanical machining and laser machining are also used [209]. The LTCC (low temperature cofired ceramics) technology based on screen printing and developed for manufacturing an electronic support for RF and microwave applications, has also been adapted to microfluidics as illustrated in figure 35(*d*) [210]. It enables simple coupling with passive electric elements made using the same techniques.

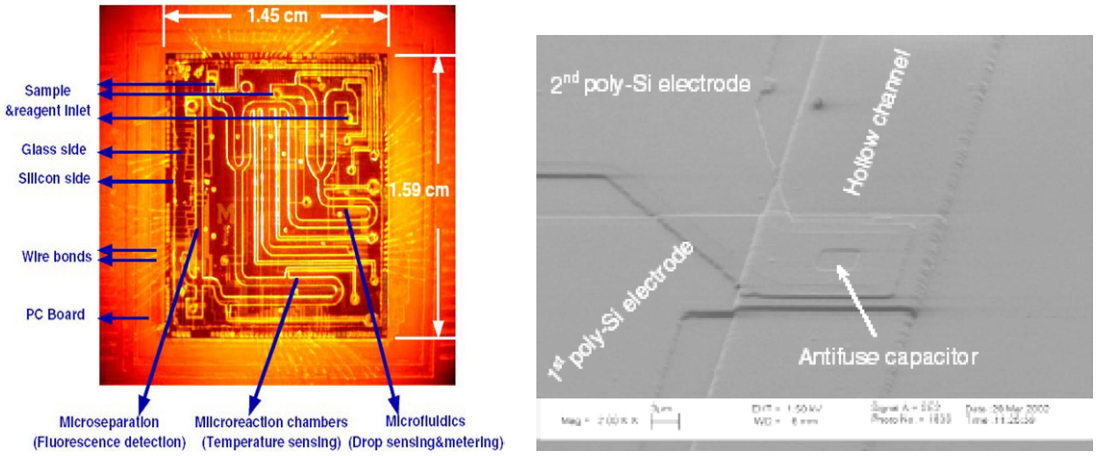
2.4. System integration

Besides the microfluidic network itself, a complete system includes other elements that interact with fluids such as electrodes, photodetectors, chemical sensors, pumps, valves, etc. Several examples of monolithic integration or transfer of a microfluidic network onto an integrated circuit have been



(*a*) (*b*) (*c*) (*d*)

Figure 35. (*a*) Ceramic structure made by replication in a PDMS mould and (*b*) pillars in the green state and (*c*) after sintering at 1280 °C [Uppsala University] [208], © 2002, with permission from Elsevier; (*d*) microfluidic circuit made using the LTCC technology [Wroclaw University] [210], © 2005, with permission from Elsevier.



(*a*) (*b*)

Figure 36. (*a*) Integrated circuit including photodiodes with a microfluidic network etched in a glass substrate [Michigan University] [211], reprinted with permission © 1998 AAAS; (*b*) channel between an emitting diode and a receiving diode [MESA+] [212].

demonstrated. Systems including photodiodes coupled with a microfluidic network made of glass have been produced as shown in figure 36 [211]. The article by Leminh *et al* describes a silicon nitride microchannel sandwiched between an emitting diode and a receiving diode [212]. Integration of polymer microfluidic networks on an integrated circuit has also been reported [213–215]. However, given the price per unit area of an integrated circuit, these solutions do not appear to be viable for applications in which long microchannels and therefore large surfaces are required. Furthermore, components such as micropumps are made by specific, unconventional approaches that are poorly compatible with integrated circuit technology.

Therefore, the hybrid integration approach appears better adapted to a lab-on-chip integration. The challenge is then to couple the different elements, for example a sensor made using the silicon technology and a microfluidic network replicated in a thermoplastic, with good alignment and without introducing additional dead volumes, and obviously at the lowest cost.

In the 1990s, Verpoorte *et al* stacked 13 silicon pieces of 22 mm by 22 mm clamped together mechanically [216]. The system integrated a 3D network of microchannels with a 200 by 600 μm section made by anisotropic wet etching, piezoelectric actuation pumps and an ISFET (ion sensitive field effect transistor) type sensor used for measuring the pH. Thanks to this stacking technology, dead volumes were low, unlike other processes in which the different modules are connected together by tubes [217]. The reversible attachment enabled individual test and maintenance of the different components. Figure 37(*a*) shows a complete system including a mixer with a pump and a flow sensor on each of the two upstream branches [218]. Each silicon component is transferred by anodic bonding onto a 'microfluidic motherboard' made of silicon/glass using the MCM (multi-chip module) approach in which bare components are mounted on a substrate comprising electrical and fluidic connections. Various systems including a pump with its fluidics and its associated electronics were made with FR4 laminate (a standard material in the printed circuit industry) covered by copper, by adapting the printed circuit technology [219].

The hybrid approach led to genuine microfluidic 'test wafers', like cards used for tests in electronics. The LioniX Company markets a platform, also based on the printed circuit technology, to which various components are connected (MATAS (Modular Assembly Technology for microTAS technology) [64]. This modular approach is also proposed by the ThinXXS Company and developed by the Fraunhofer

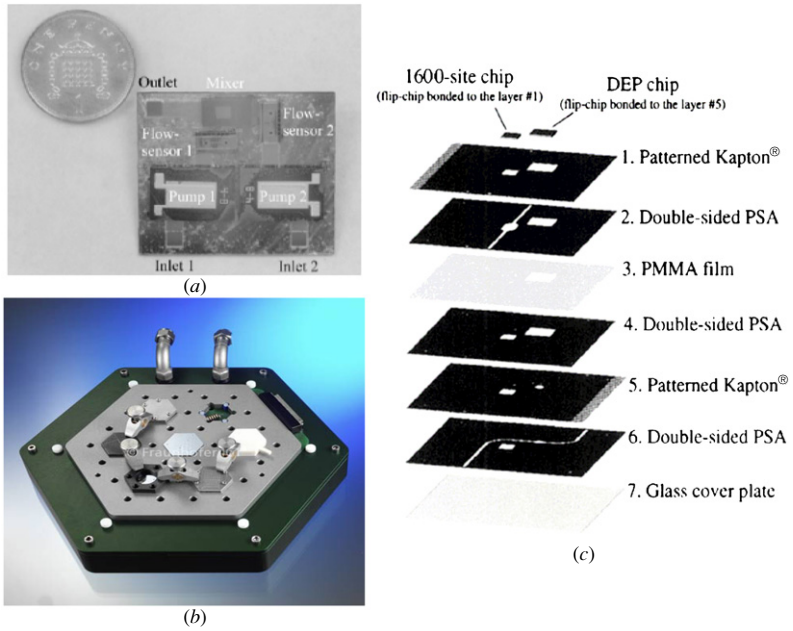


Figure 37. (*a*) Microfluidic motherboard made of a silicon/glass technology [Southampton University] [218]; (*b*) FAMOS modular platform [FhG] [221] used with permission; (*c*) heterogeneous lamination and chip transfer by flip-chip [Nanogen] [224], © 2002, with permission from Elsevier.

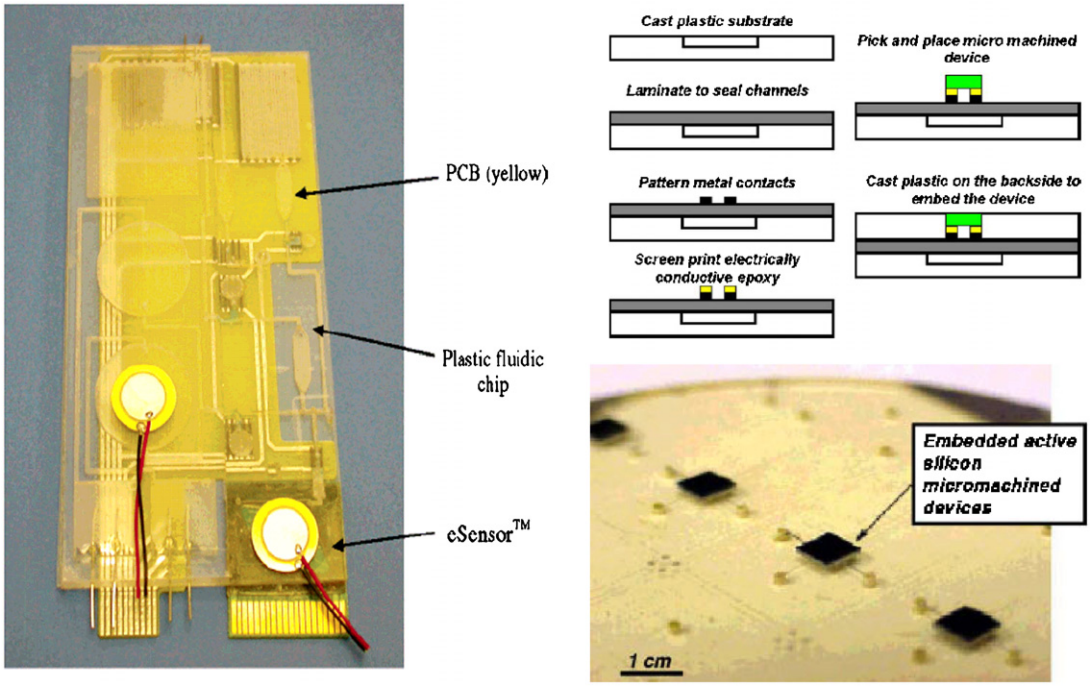
Gesellschaft (FhG) through the FAMOS (Fraunhofer Alliance Modular Microreaction System) platform shown in figure 37(*b*) [220, 221]. The connections are then crucial so as to prevent dead volumes.

The use of polymer technologies for manufacturing the microfluidic network can once again avoid the disadvantages of silicon systems (optical properties, incompatibility with electrokinetics) and glass (price of the material, difficulty of etching). It introduces many other frequently mentioned advantages (variety of materials, low process cost) and also has other advantages, particularly in terms of integration: relatively low working temperatures, planarizing properties and photosensitivity for many resists .... One strategy consists of coupling plastic microfluidic cartridges with external macroscopic instrumentation, as for example in the case of the i-STAT portable blood immunotest instrument or the system described by Tamanaha *et al* [222, 223].

Another strategy consists of integrating all the functions on one substrate. The approach of the Nanogen Company is shown in figure 37(*c*) [224]. It consists of stacking different 76 mm by 76 mm prestructured layers by lamination. Kapton is a polyimide marketed by Dupont de Nemours that can be used as an electronic substrate, particularly for manufacturing flexible circuits (flex technology). Therefore the integration of electrodes and the transfer of components onto this type of substrate are straightforward. In this case, two silicon chips are transferred onto the Kapton levels 1 and 5. The level-to-level bonding is guaranteed by PSAs (pressure sensitive adhesives) in which the fluidic networks are drawn. Although the approach is very attractive in terms of integration, it can be noted that the heterogeneity of the materials used can be inconvenient, for example for an electrokinetic actuation or for any other application for which the control of the surface properties would be important. Furthermore, the precision of the level-to-level alignment is not given.

A hybrid integration solution was also demonstrated by a Motorola team [225]. The system presented in figure 38(*a*) couples a network of microchannels with sections with millimetre dimensions machined from polycarbonate and mounted on a printed circuit. Electrodes are used to heat some areas to perform functions such as pumping (expansion of an air pocket) or opening/closing a microchannel (melting/solidification of wax in a channel, in one step). These electrodes were also used for another pumping principle (generation of gas by electrochemistry) and for detection, after grafting DNA strands terminated by thiol functions. Piezoelectric elements were integrated onto reaction chambers to improve the mixing performances. Therefore the dimensions remain large, alignment precision is poor and integrated functions are limited by the technology (based on the use of electrodes only). Wagler *et al* have demonstrated integration of a PDMS microfluidic component on a silicon substrate comprising 6200 electrodes controlled by a logic component mounted in a BGA (Ball Grid Array) package [226]. Details on alignment of the PDMS part on the substrate are not given. Sethu *et al* also present an elegant technology shown in figure 38(*b*), used to combine a silicon chip and a microfluidic network made by casting, using an epoxy resist instead of PDMS [167]. It is used to mount components, for example diodes, on a polymer substrate comprising a microfluidic network. However, it does not allow bringing the component into direct contact with the fluid, for example such as the sensitive layer of an ISFET with the solution to be tested.

More recently, LAAS-CNRS has proposed an approach that can overcome this difficulty [227]. A schematic representation is given in figure 39. As the first step, the silicon



(*a*) (*b*)

Figure 38. (*a*) Card combining a printed circuit, a plastic fluidic network and piezoelectric elements [Motorola] [225], reprinted with permission, © 2004 American Chemical Society; (*b*) transfer of bare chips onto a microfluidic component obtained by casting an epoxy resist [Michigan University] [167], © 2004, with permission from Elsevier.

chip is reported onto a printed circuit board (PCB) that provides electric contacts for connection to external instruments or power supply (1(*a*)). Plots of conductive ink are screen-printed in order to create inter-level electrical vias and this assembly is planarized with a polymer (1(*c*)), also using a screen printing technology. Then, conductive tracks are patterned in order to connect the silicon chip to the PCB pads through the previously deposited vias (1(*d*)). Thanks to the planarization step, photolithography and standard microelectronic processes can be used if high miniaturization and precision are needed.

Finally, as shown in figure 39(2), the microfluidic network can be reported onto this structure or realized level by level as already depicted here above [198] and additional devices can be mounted on the back side of the PCB. A simple microsystem integrating a silicon sensor in a SU-8 channel is shown in figure 39(3).

The fluidic connection between the microsystems and the macroscopic world is also challenging. Several solutions were proposed depending on the technology used. For PDMS, sleeves were simply inserted in holes formed in the chip, as can be seen in figure 26(1), with silicone providing the seal. Other systems are necessary to connect a rigid microfluidic network. The Upchurch Company market connectors that bond to the surface of glass or silicon chips [228]. Several solutions were also proposed to prevent excessive dead volumes [229, 230].

3. Microfluidics and applications

3.1. Advantage of miniaturization

The concept of μTAS, later extended to the lab-on-a-chip concept, was introduced in 1990 to mitigate the deficiencies of chemical sensors, particularly in terms of selectivity and life time [32]. In his founding article, A Manz mentioned the example of blood. If a species with a concentration of the order of 10^{-5} mol L^{-1} has to be detected, the sensor must reject at least one hundred species with a higher concentration. Therefore preprocessing would be necessary. The concept is to carry out the preprocessing steps automatically, eliminating most undesirable species; the sensor does not need to be highly selective. The concept of the total chemical analysis system (TAS) introduced at the beginning of the 1980s relates to automation of operations in analytic chemistry [231]. Sampling, transport of samples, possible chemical reactions, separations and detection are automated.

Integration of this concept onto a chip can also very strongly reduce the consumption of chemicals that may be rare, expensive and/or polluting. Similarly, it reduces the production of waste. Automation of procedures illustrated by the DNA analysis station presented in figure 40(*a*) and marketed by the Caliper Company, increases analysis rates and reduces the risk of error due to the human factor [232]. Furthermore, these products enjoy the advantages of prices for mass production processes. Miniaturization also provides a means of making these systems portable.

Cost and size reductions are not the only advantages; performance gain is also important. For example, an immunoassay was carried out in less than 25s while more than 10 min are necessary under classical conditions [233]. The i-STAT portable system shown in figure 40(*b*) used in a medical environment, can be used for various analyses starting from two drops of blood in a few minutes instead of a few hours if the sample has to pass through a laboratory. The microreaction field initiated by institutes such as FzK

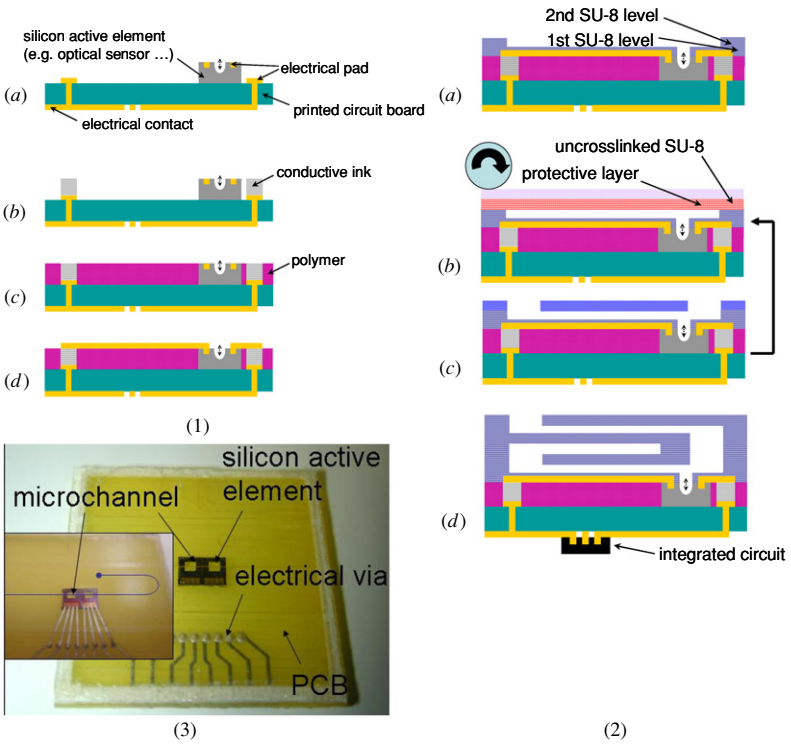


Figure 39. Hybrid integration process developed at LAAS-CNRS (1) of the silicon chips and (2) level by level realisation of the 3D microfluidic network. (3) hybrid microsystem integrating sensors in a SU-8 microfluidic network.

(*a*) (*b*)

Figure 40. Examples of commercial labs-on-chip: (*a*) automated CE system on Calliper gel [232] used with permission; (*b*) portable i-STAT system for analysis of blood in a medical environment [222] used with permission.

and the Institut fuer Mikrotechnik Mainz (IMM) (Mainz Microtechnology Institute) benefits from these effects [206]. Thus, separations are faster and have a better resolution. In CE, the reduction of the section can make it possible to use stronger electrical fields without increasing the electrical current, which is a source of heat and therefore greater longitudinal dispersion. In liquid chromatography, it reduces the flow without changing the velocity on which the efficiency depends, which provides a means of directly coupling a mass spectrometer to the column.

The precision of the manufacturing method itself may be an advantage as shown by F Regnier at Purdue University with collocated monolithic support structures (COMOSS), stationary phase obtained by photolithographic processes, for which the tight distribution of dimensions enables limitation of Eddy diffusion [70]. Furthermore, microtechnologies and nanotechnologies can be used to perform new functions such as cyclic CE, in which integration provides a means of eliminating dead volumes or enabling DNA entropic trapping in microchannels alternating micrometric and nanometric zones [234, 235].

3.2. Example applications

3.2.1. Analytical chemistry. Even if analytical chemistry is not an application in itself but is rather a tool, it is at the heart of many labs-on-chip. The system used by J Harrison in 1992 during the first separation on chip by CE is described in figure 41 [236]. The chip was manufactured from glass by wet etching and thermal bonding and integrated platinum electrodes. The external dimensions were 14.8 cm × 3.9 cm × 1 cm. The width of the microchannels 2 and 3 was 30 μm, the width of the channel 1 was 1 mm, and the depth of all of them was 10 μm. Firstly, the sample that was a mixture of fluorescein and calcein, was introduced with a syringe through the reservoir 2. The buffer solution in which separation took place was introduced through the reservoir 1 and at the same time rinsed the excess sample in the separation channel, the reservoir 2 being blocked. The injection phase then began by applying a voltage of 250 V between reservoirs 2 and 3, for 30 s. Finally, separation was done by applying a voltage of 3000 V between reservoirs 1 and 3. Detection took place at the end of channel 3 by laser induced fluorescence (LIF), in other words by exciting the compounds with a laser and collecting the information with a photo multiplier tube (PMT). The corresponding electrophoregram is also shown in figure 41. It shows two perfectly resolved peaks, in other words a separation made.

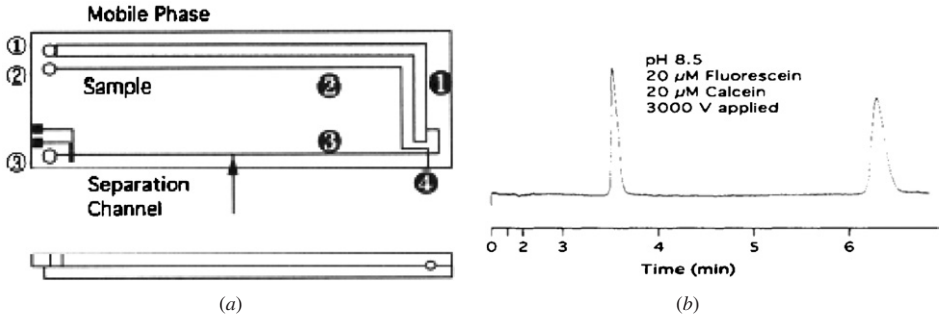


Figure 41. (*a*) Geometry of the first CE chip; (*b*) electrophoregram for separation of a fluorescein/calcein mixing [236], reprinted with permission, © 1992 American Chemical Society.

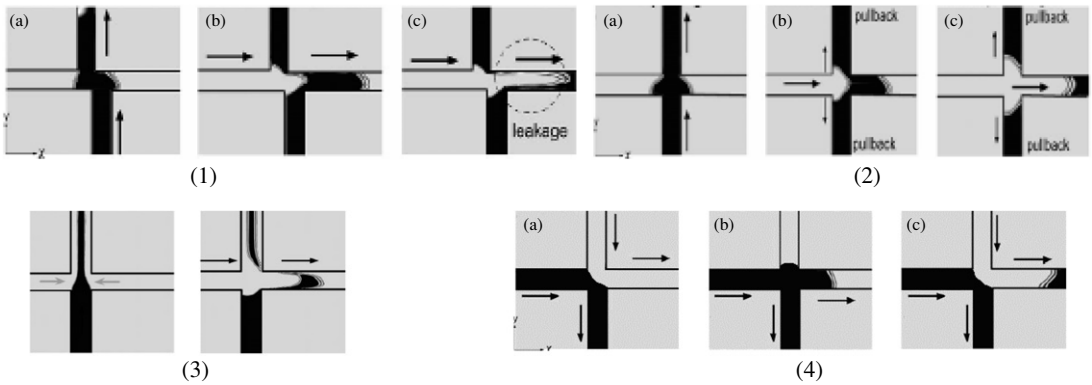


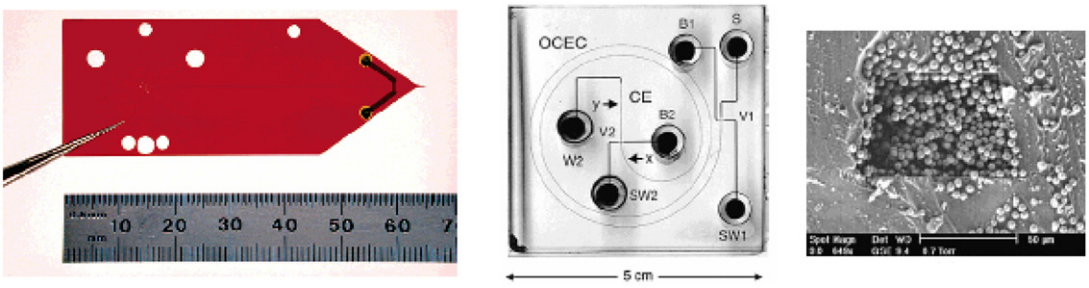
Figure 42. Different types of injection (1) conventional double T injection; (2) injection with 'pullback'; (3) pinched injection; (4) gated injection [240], reproduced with permission.

A large amount of work was done later on similar systems [237–239]. Several methods have been developed to improve the control of the injected volume, and some of these methods are described in figure 42 [240]. The most conventionally used injection principle consists of injecting the sample into a microchannel perpendicular to the separation channel as can be seen in (1, *a*), and then changing the voltages so as to send the sample contained at the intersection into the separation channel. With this technique, the injected volume depends on the geometry of the intersection (frequently a single cross or a double T as shown in figure 42(*a*)) defined during the design of the masks and not on time as in the first work done by D J Harrison *et al*. A first problem with this technique is leakage of the sample during separation as shown in (1, *c*). One means for avoiding this effect is to pullback the liquid from lateral microchannels during the separation. A second problem is the lateral diffusion that is difficult to control as observed in (1, *a*), and which is a source of poor reproducibility. One solution is electrokinetic focusing as shown in figure 42(*c*) for mixing. Another technique called the gated technique consists in circulating the buffer solution and the sample in the configuration shown in (4, *a*). Since conditions are laminar, the liquids do not flow. The buffer inlet reservoir is then brought up to a floating potential for a short moment, before returning to the initial conditions. The injection volume then depends on the switching time.

One can note the important length of channels frequently necessary for this type of application. One solution for reducing the size of the chip is to design serpentine separation channel. However, the addition of turns generates dispersion. One solution to minimize this effect is to reduce the channel width in the turn [241].

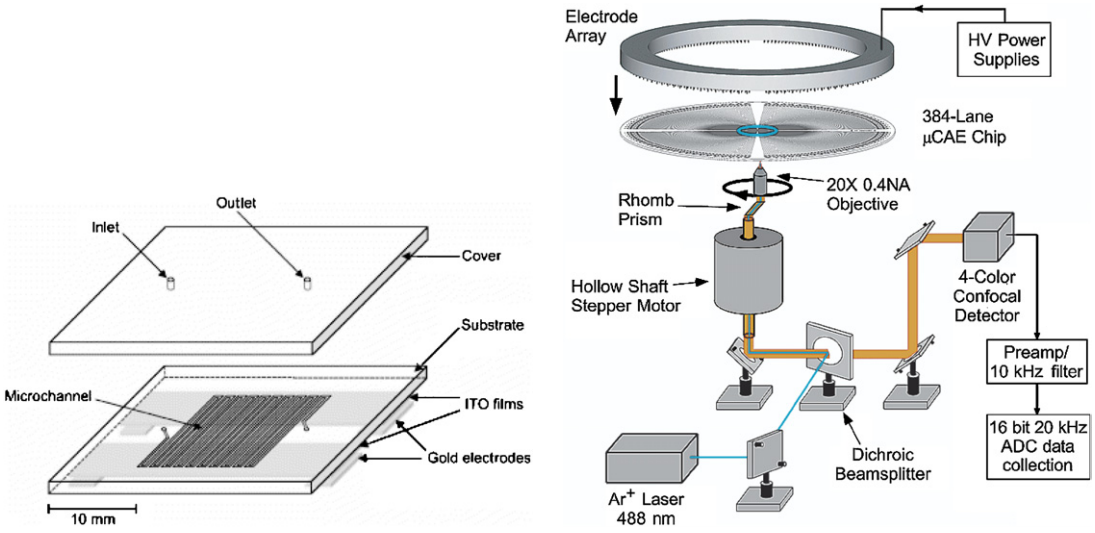
Optical detection may also be integrated [242]. Reference [243] describes a portable CE system and its power supply and electrochemical detection. This detection type is less sensitive than detection by LIF but is easier to integrate and is used to detect non-fluorescent species. It has been demonstrated that a measurement can be made by capacitively coupled conductivity and therefore with no contact with the liquid, so as to avoid problems with the formation of bubbles [244].

Very little work has demonstrated the integration of high performance liquid chromatography (HPLC) on a chip. Recently, Agilent has developed a chip made by laminating polyimide comprising preconcentration and HPLC separation columns and the coupling spray nozzle with a mass spectrometer [245]. Pressures of up to 200 bars have been applied. The magnitudes of the pressures involved are such that although HPLC is predominant in the analysis field, the integration of capillary electrochromatography (CEC) systems is much more used [246]. In this technique, the fluid actuation is governed by electroosmosis, and no longer by pressure. Another advantage compared with HPLC is due to the flat profile of the electroosmotic flow that generates less dispersion than the parabolic profile of flow under pressure. Ramsey's group described the first CEC system on a glass chip in 1994 [247]. An example of a 2D separation system, i.e. combining different physicochemical principles, comprising a CEC followed by CE is shown in figure 43(*b*), together with an example of stationary phase integration [248, 249].

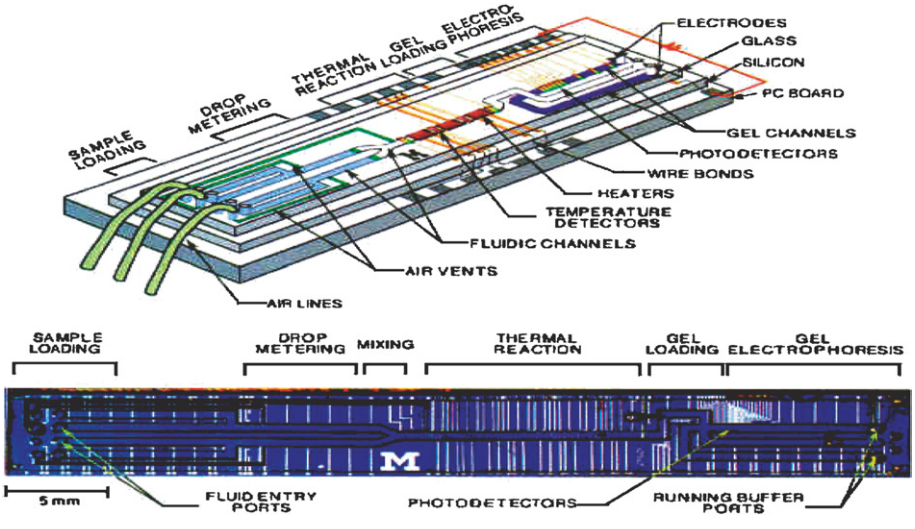


(*a*) (*b*) (*c*)

Figure 43. (*a*) Chip integrating an HPLC column and the coupling plume with a mass spectrometer [Agilent] [245], reprinted with permission, © 2005 American Chemical Society; (*b*) system used for 2D separations [Oak Ridge National Laboratory] [248], reprinted with permission, © 2001 American Chemical Society; (*c*) section through a microchannel with its stationary phase [Neuchâtel IMT] [249], reprinted with permission, © 2002 American Chemical Society.



(*a*) (*b*)



(*c*)

Figure 44. (*a*) System integrating PCR thermal cycles [Tokushima University] [275], © 2002, with permission from Elsevier; (*b*) detection by LIF with a rotary head of a high flow sequencing microfluidic system [Berkeley] [251], reprinted with permission, © 2002 American Chemical Society; (*c*) lab-on-chip including preparation of the sample, PCR and separation [Michigan University] [211], reprinted with permission © 1998 AAAS.

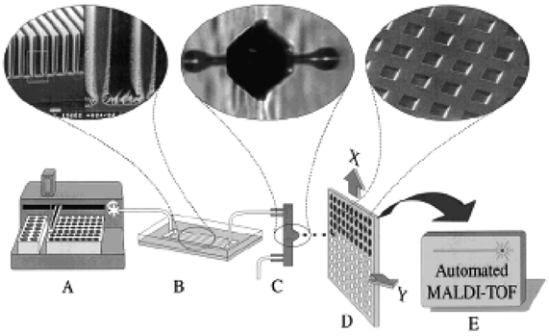


Figure 45. Automation of a protein analysis procedure using MALDI-TOF-MS [Lund University] [253], reprinted with permission, © 2000 American Chemical Society.

3.2.2. Genomics. Many lab-on-chip applications have been developed in genomics, under the emphasis of the human genome project and sequencing needs [250]. One of the key tools is polymerase chain reaction (PCR) that copies and amplifies the required section of a DNA strand sampled before the analysis. This technique requires precise thermal cycles after preparation of DNA. Therefore miniaturization of volumes provides a means of reducing the time for the operation. Figure 44(*a*) shows one example of an integrated thermal cycle without sample preparation. The assembly was made transparent by the use of glass and ITO electrodes. The sample was transferred alternately from an area at 94 °C to an area at 67 °C. Within the framework of high flow sequencing, Ramsey's team has fabricated a system on a 20 cm diameter circular glass plate comprising 384 separation columns by CE on gel operating in parallel, and an associated rotary readout instrument presented in figure 44(*b*) [251]. Its use has been demonstrated for early detection of a disease in a population. Figure 44(*c*) shows another example of a system including the DNA sample preparation step, the PCR and CE on gel [211].

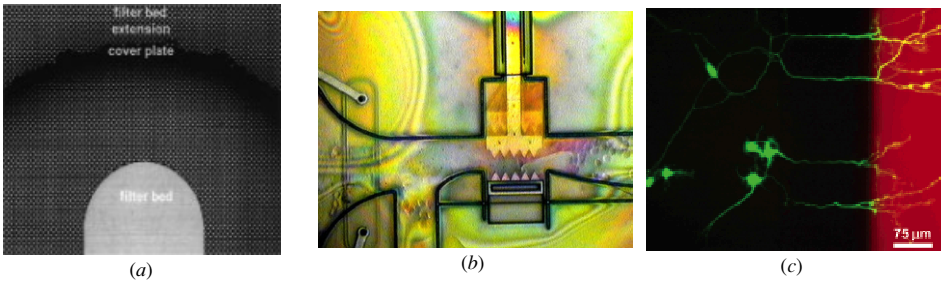
3.2.3. Proteomics. While genetic analyses became increasingly routine operations, efforts have been made and are still being made on analysis of proteins. Its traditional approach includes extraction from cells, separation by electrophoresis on gel, digestion and analysis by a mass spectrometer. These operations are slow and tedious. A system was made to perform the digestion, separation and analysis steps [252]. The operation that normally takes hours is done in a few minutes. Although CE is frequently used for separation, capillary electrochromatography (CEC) is better adapted to the analysis of complex protein samples [246, 248] Coupling of CEC or HPLC with a mass spectrometer as shown in figure 43(*a*) has also been reported [245]. Another conventional technique in proteomics consists of analysing a plate of microvials filled with proteins digested by a particular mass spectrometry technique (MALDI-TOF MS—matrix-assisted laser desorption/ionization time-of-flight mass spectrometry), directly. The system shown in figure 45 includes pre-treatment (A), digestion (B), ejection (C) onto the spectrometer target (D) and the analysis (E) [253]. Although the process normally includes several manual steps, and digestion alone lasts between 6 and 24 h, the analysis with this automatic system is completed in only 3 h and a half.

3.2.4. Cellomics. Microfluidic systems have also been developed for the manipulation of cells, with an even greater degree of complexity in the biological scale [254, 255]. The dielectrophoresis phenomenon was used to manipulate cells on a substrate [256]. An example of a filter for cell capture composed of 3.4 μm wide and 10 μm high microchannels obtained by RIE in quartz is shown in figure 46(*a*) [257]. Another capture system is shown in figure 46(*b*) [258]. The cells are immobilized without being damaged, by teeth to enable various operations. The long-term objective is to replace the teeth by needles so as to inject DNA, RNA or proteins.

One technique that allows rapid analyses of populations of cells is flow cytometry. This technique consists of forming a train of cells by focusing them by hydraulics or by electrokinetics after marking and then counting them. Reference [259] shows an example of integration and counting Escherichia Coli bacteria.

There are other applications of microfluidics in other fields, such as tissue engineering [260]. Figure 46(*c*) shows an example of neurone growth in PDMS microchannels [261]. Axons are aligned due to the presence of microchannels that cannot be seen on the photograph. This type of tool is useful for research in neuroscience, the example mentioned being the struggle against Alzheimer's disease.

3.2.5. Imunnoassay. The term 'immunoassay' denotes the different dosing methods that take advantage of the capacity of antibodies and antigens to bond specifically. This is a



(*a*) (*b*) (*c*)

Figure 46. (*a*) Filter [Purdue University] [257], reprinted with permission, © 1999 American Chemical Society and (*b*) mechanical cell capture unit [Sandia] [258], used with the permission of Sandia National Laboratories; (*c*) growth of neurones controlled by a microfluidic network [California University] [261], reprinted with permission, © 2003 American Chemical Society.

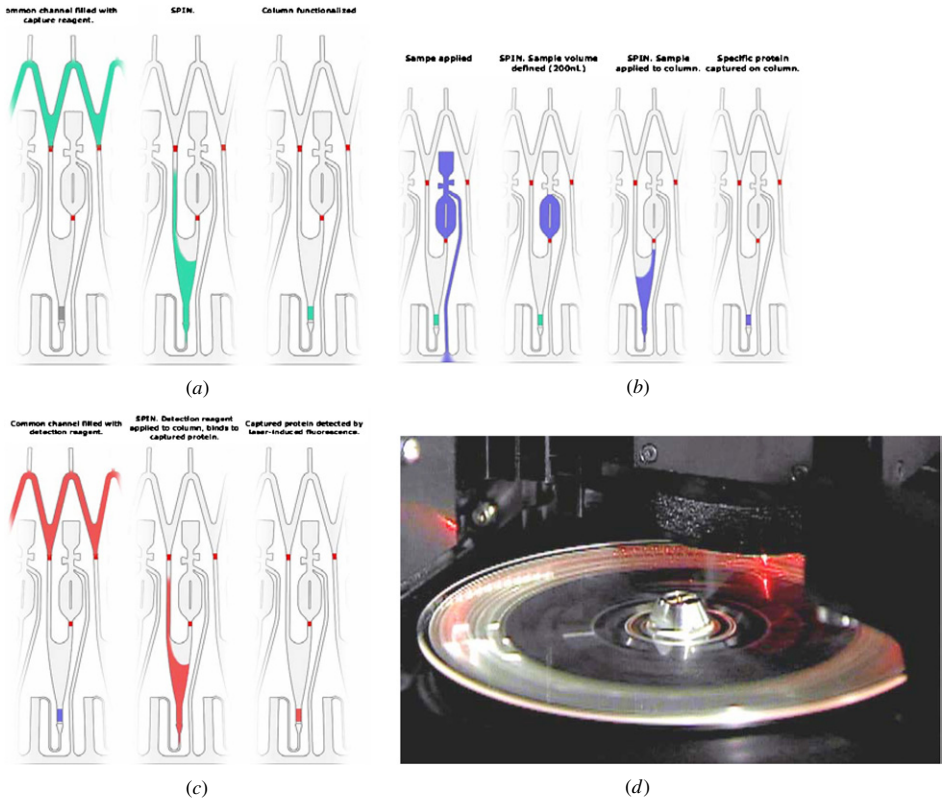


Figure 47. ELISA assay integrated on a CD format with actuation by centrifugal force (*a*) fixation of antibodies; (*b*) introduction of the antigen; (*c*) reaction with the tracer antibody; (*d*) readout by LIF [Gyros] [156], reproduced with permission.

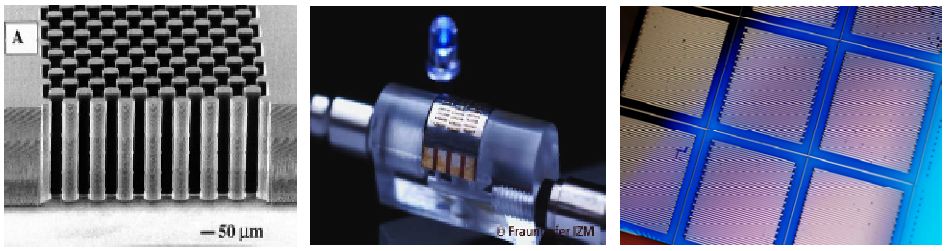
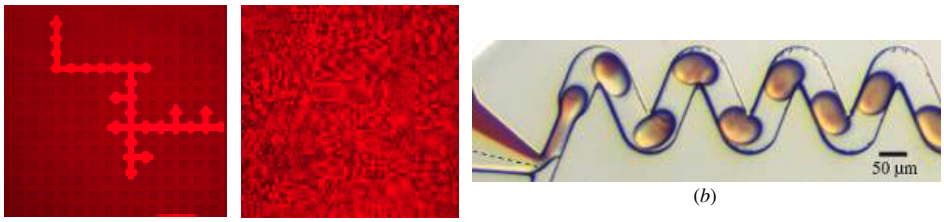


Figure 48. (*a*) Silicon columns of a catalytic reactor [MIT] [263] (© 2002 IEEE); (*b*) microfuel cell supplying a LED [Fraunhoffer IZM, Berlin] [264], © 2004, with permission from Elsevier, (*c*) microfluidic cooling of an integrated circuit [GeorgiaTech] [265].

standard tool for diagnostic and understanding of diseases [262]. These analyses are repetitive and laborious. Once again, microfluidics provides a means of automating this task and reducing reaction times. For example, the Enzyme Linked ImmunoSorbent Assay (ELISA) is widely used to dose a protein in a liquid. The so-called sandwich dosing technique consists firstly of fixing antibodies capable of specifically bonding the searched antigen on the surface of wells. The solution to be dosed is deposited in the wells. If the searched antigen is present, a specific bond will be created with the antibodies. The second antibody is introduced, forming the tracer antibody that will bond specifically with the antigen. The assembly is finally rinsed. Detection may take place for example by optical readout if the tracer antibody was marked with a fluorescent compound [156].

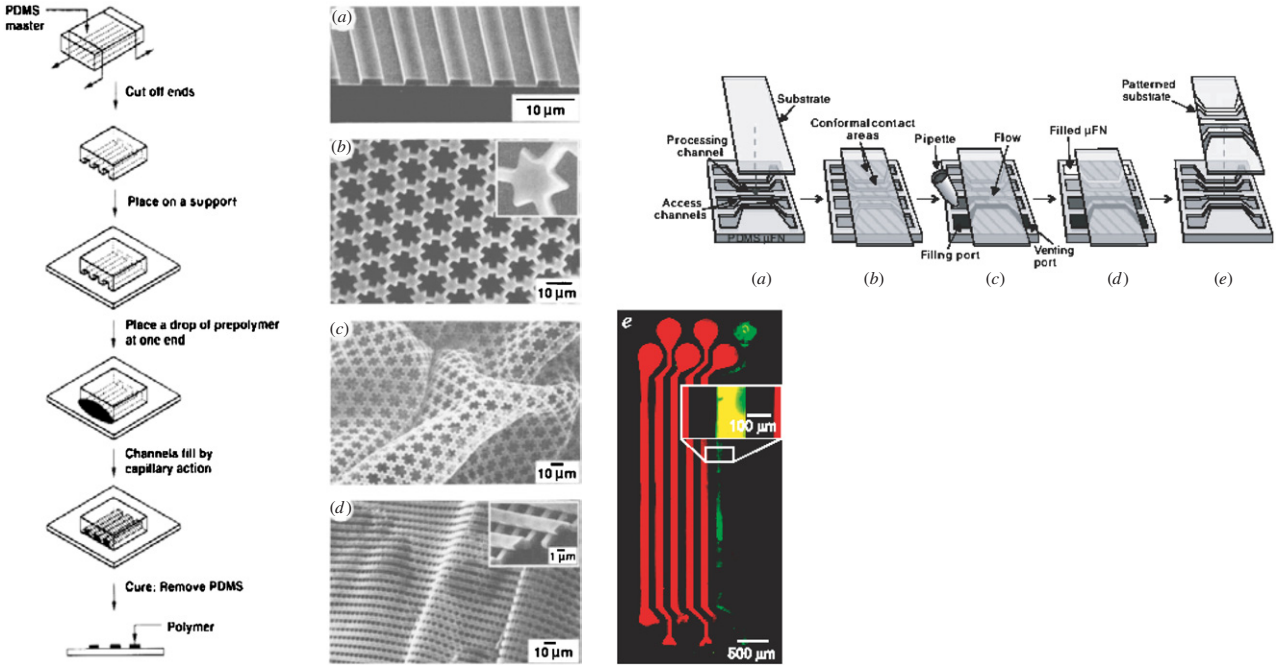
An ELISA assay integrated in a CD format is marketed by the Gyros Company. The principle of the analysis is described step by step in figure 47. The dark zones are hydrophobic valves; flow is only possible beyond a limiting value of the applied pressure, controlled by the rotation speed of the CD. This principle is used to inject precise quantities of liquid. In step (*a*), the solution containing capture antibodies is deposited and then injected in a column containing beads functionalized with streptavidine on which the antibodies will be fixed. A precise dose of the substance to be analysed is then introduced and circulates in the column (*b*). Finally, the tracer antibody is injected (*c*) and is read by LIF (*d*).

3.2.6. Microreaction. The possibility of faster heating and mass transfers in microfluidics open up many opportunities in the field of reactions by enabling efficiencies higher than with traditional reactors [204–206]. Furthermore, reactions under difficult conditions using dangerous products, or for example with a risk of explosion, may be done given the



(*a*) (*b*)

Figure 49. (*a*) Study of percolation with a micromodel [Purdue University] [266]; (*b*) formation of drops/microreactors in a two-phase flow [University of Chicago] [272].



(*a*) (*b*)

Figure 50. (*a*) MIMIC technology [Harvard] [159], reprinted by permission from Macmillan Publishers Ltd © 1995, (*b*) μFN technology [IBM] [274].

volumes involved; the incident may easily be contained if there is a failure on a reactor. In the article by Losey *et al*, the execution and operation of a reactor for catalysis and mixing of a solid phase and a liquid phase was demonstrated [263]. This reactor was made using the silicon technology and integrated porous columns for the catalysis that can be seen in figure 48(*a*) before electrolysis in HF, and heating and temperature control electrodes. Microfuel cells can also be included in the field of microfabrication. An example is shown in figure 48(*b*) [264]. This flexible structure is made by successive laminations of stainless steel films and polyimide films structured by RIE. Another application that does not form part of the microreaction but that also benefits from the high capacities for heat exchanges is cooling of integrated circuits [265]. For example, the low temperature thermodegradable sacrificial layers technique used at GeorgiaTech and described above has been integrated at the end of manufacturing at the back face of components as shown in figure 48(*c*).

3.2.7. Research tools. Micro- and nano technologies are also a wonderful means of studying physical phenomena at a submillimetric scale. Geologists used micromodels at the beginning of the 1980s to study problems such as percolation in porous environments [266]. One example result is shown in figure 49(*a*). The size of pads is 7 μm. The precision provided by the technological tools enables comparison with theoretical models. In [267], a microchannel including temperature sensors is used to study the laminar flow of liquid. A similar approach was adopted for the study of gas flows in a structure integrating pressure sensors [268]. Flow in nanochannels is also an interesting topic, as illustrated by studies on capillarity or the recent experiment with a nanofluidic transistor at Berkeley in which the gate voltage controls the electrokinetic flow [269–271]. Another study example is two-phase oil/water flows illustrated in figure 49(*b*), used particularly for production of drops forming effective microreactors [272].

3.2.8. Lithography. For this final application mentioned in this state-of-the-art, microfluidics is becoming a tool at the service of the micro- and nanotechnologies. The MIMIC (MIcroMoulding In Capillaries) technology described in

figure 50(*a*) was introduced by G M Whithesides in 1995 [159]. The objective is to obtain a polymer microstructuring technology. The principle is to transfer a PDMS microfluidic circuit onto a substrate, to circulate a low-viscosity prepolymer on it by capillarity and then to cross-link. Examples of PMMA structures on a substrate and detached from the substrate are presented. The structure (*d*) was made between two pieces of PDMS structured in the same way as in case (*a*). The technique was validated with different polymers and substrates. One of the obvious limitations is that the prepolymer has to enter the fluidic circuit by capillarity. The principle of the μFN (MicroFluidic Networks) technique presented by IBM in 1997 was to modify a substrate locally by circulating a liquid inside a PDMS microfluidic network [160]. As for the MIMIC or lift-off technology, this technology avoids photolithography and etching after deposition. The example of a localized fixation of immunoglobulin B was shown and an ELISA assay was validated. Grey-scale photolithography was also performed using microfluidic photomasks. In this last example, the opacity of the photomask can be tailored by the concentration of dyes in a microchannel [273].

4. Conclusions

Many technological tools are available for manufacturing microfluidic networks. There is no standard process but there are many different methods depending on the application, the geometry of the channel (length, minimum dimension), the required integration type (single microchannel or three-dimensional network integrated with electronics, sensors, etc). This popularity has been a driving force over the last 10 years for the application and development of micro- and nanotechnologies with new materials such as polymers. There are a very wide variety of applications of technological tools based on microfluidics, including obvious interests such as speed, automation, portability, low quantities of consumed and waste products, better performances, etc. However, making complex three-dimensional systems is still a challenge:

- most existing labs-on-chips are based on macroscopic instrumentation around a microfluidics core,
- assembly technologies only have poor precision and they further limit possibilities of complexifying polymer structures,
- polymer technologies are still difficult to make compatible with integration of active components for example made of silicon.

The microfluidic network may, for example, be placed directly on an integrated silicon circuit. However, as has been seen throughout this review, the surface of a microfluidic circuit can be very large (a few tens of square centimetres), particularly for analytical chemistry applications in which microchannels with dimensions equal to a few tens of centimetres may be required. Considering the price of machining of silicon per unit area, it is easy to understand that this system on chip (SOC) approach does not appear to be viable. Another possibility is to build the microfluidic network on a hybrid system, in which bare integrated circuits can be made with different technologies, and are transferred onto a substrate providing electronic connections (multi chip module—MCM-approach).

It is becoming obvious that complete low cost silicon/polymer hybrid integration solutions must be explored, to obtain the full benefits of the advantages of integration.

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