Silicon and glass micromachining for μTAS

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1. INTRODUCTION

The controlled manipulation at micron-scale of liquids by mechanical actuation, electric or magnetic fields, pressure or surface tension has been one of the essential elements for the rapid development of μTAS over the past decade. Although commercially successful μTAS products are mostly realized in plastics, glass or ceramics [1], in many cases the first demonstration of the functionality of the microfluidic/analytical device was done in silicon. For example, the classical 'μTAS-avant-la-lettre' micro gas chromatograph by Terry et al. [2], the first prototype of a micro Polymerase Chain Reaction device by Northrup et al. [3], the first attempts to do liquid chromatography on a chip [4], the first Flow Injection Analysis on a chip [5] and the first controlled pore-size microfilters [6] were all based on silicon micromachining. The main reason for the success of silicon technology also in the lab-on-a-chip field is that much more than for any other material, the properties of silicon are well-characterized, while an incredibly large 'toolbox' of machining techniques nowadays is available, originating from the developments in the electronic industry [7]. This implies that it is possible to fabricate virtually any geometrical structure in silicon with very high precision. Other reasons to use silicon are the mechanical properties (hard, strong), electrical properties, CMOS and cleanroom compatibility (no pollution), material purity, and the possibility to passivate it with chemically well-known and relatively inert insulating layers of silicon dioxide. An additional advantage of the use of silicon is the straightforward realization of nanometer-size structures, offering new opportunities for exploration of nanofluidic phenomena.

This chapter will focus on micromachining of fluidic structures in silicon, and several examples of demonstrated functionality of devices in silicon will be given. Some of these demonstrators use a combination of glass and silicon, and several methods exist for bonding the two. This leads us to the shaping of microstructures in glass, with discussions how the batch microfabrication

processes, originating from microelectronic industry, are used to structure glass plates. Finally, the integration issue will be treated, since a true μTAS will consist of several components combined in a clever way to establish the functionality of a miniaturized lab.

2. OVERVIEW OF MICROMACHINING METHODS

2.1. Batch fabrication philosophy

A large family of microfluidic (and MEMS) devices has been developed, starting from the primary insight that the development philosophy that made semiconductor microelectronic industry to such a great success, should also be applicable to non-electronic problems. According to Menz [8], this success is due to the following recipe of 4 essential steps:

1. "Computer aided design": design, optimisation and simulation of the micro (electronic, optic, fluidic, etc.) circuit, and in most cases also the corresponding fabrication process, can be done on a computer. The design tools for microelectronics are so sophisticated that in the development of new circuits in most cases expensive and time consuming testing is not required.

2. Transfer of the designed circuit pattern to a substrate by means of optical imaging (photolithography). The patterns are transferred in series to a set of "masks" and subsequently imprinted in parallel on a substrate.

3. Batch fabrication: processes are applied that simultaneously treat the surface of a large number of substrates, or at least a large number of equal areas on one substrate, therewith minimizing the variation in process quality. Consequently, the technological and metrological costs of a process step are distributed over thousands of components.

4. Linking a large number of identical components with high packing density to get to an intelligent system of high quality. Probably this was the decisive step for the continuing development in microelectronics. Particularly in this step lies the power of the technology: due to the integration possibilities, this technology offers unlimited opportunities that are virtually impossible to achieve with other fabrication processes.

The processes that will be described here are selected based on above philosophy, mainly because such processes (with the exception of processes like hot embossing and injection molding that are used for polymer microfabrication, discussed in another chapter) offer the best potential for large volume production. Nevertheless, the reader should be aware that a growing number of microfabrication tools has become available, based on a "fine-mechanical" approach of piece-by-piece fabrication, like laser ablation and electrochemical discharge drilling, see e.g. [9-11] as well.

2.2. Silicon micromachining

2.2.1 Introduction

An extensive overview of the silicon micromachining methods to be described below can be found in ref. [12]. Here we briefly summarize the basic principle of each method, while in the next section a number of examples, to illustrate the feasibility of the techniques, will be given.

2.2.2 Bulk etching

An often-used method for micromachining of silicon is anisotropic wet-chemical etching. This process, in which silicon is dissolved in a concentrated alkaline solution (e.g. 25 wt-% KOH in water) at temperatures around 70°C, exploits the crystallographic properties of defect-free single-crystalline silicon substrates.
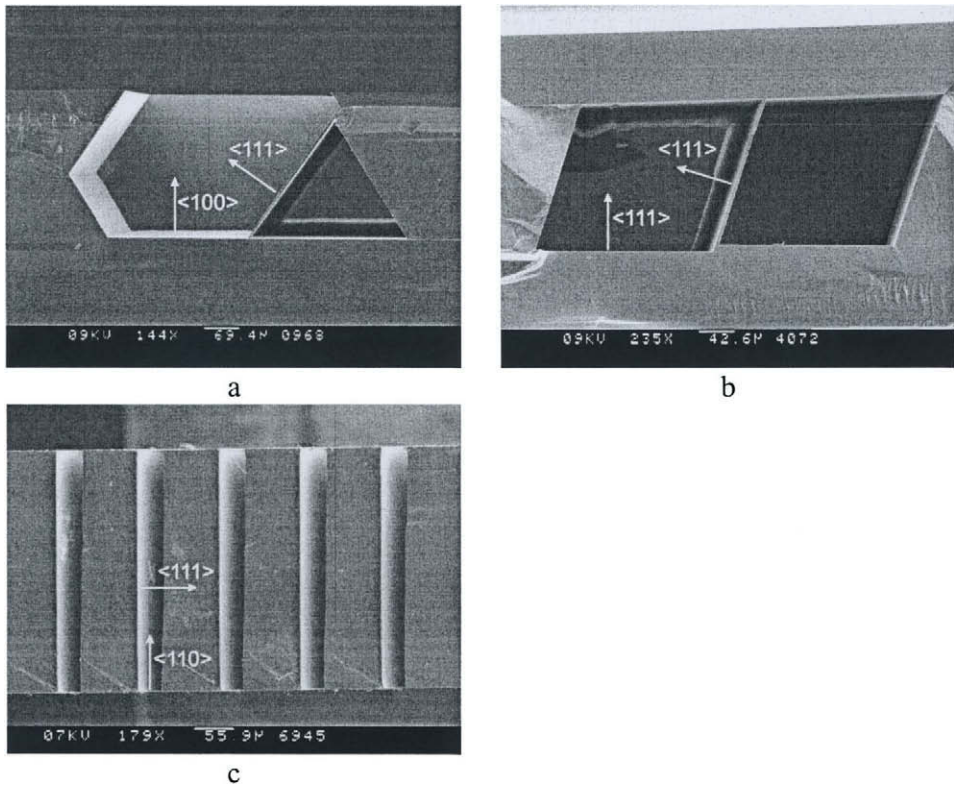

a

b

c

Fig. 1. Structures etched in a) {100}-silicon, b) {110}-silicon and c) {111} silicon. The channels in the {111} wafers were pre-etched with reactive ion etching. The anisotropic wet chemical etching step afterwards will create the channels, bounded by {111}-planes. The channels of figure a) and c) were created without pre-etching.

The structures that develop in this process are bound by crystal planes with the highest atomic density, the $\{111\}$ planes. Depending on the selected crystallographic orientation of the wafer surface and the design of openings in the masking layer (usually a thin film of SiO_2 or Si_3N_4), several shapes can be fabricated with this method.

Fig.1 shows structures, etched in silicon with different wafer crystal orientations, which may serve as fluidic channels. Examples of the application of such structures are channels for liquid chromatography [4], enzyme microreactors [13] and integrated chip cooling [14]. The method was most frequently used for the fabrication of membranes for pressure sensors. Such membranes, however, may also serve as check valves or membranes in micropumps [15]. If the pyramidal features that are obtained in Si $\{100\}$ wafers are etched through the substrate, structures are obtained that can be applied as ink jet nozzles [16]. Examples of more "creative" etching of rosettes in $\{100\}$ [17] and check-valve spring structures in $\{111\}$ silicon [18] are shown in figure 2.

A drawback of anisotropic etching is the limited design freedom in the fabrication of channels with sharp bends. Because of the anisotropic etching rates, the exact shape, as defined by the mask will never be obtained. Convex corners tend to develop towards a shape, limited by the fastest etching planes, and concave structures will always end up with $\{111\}$-oriented surfaces. Corner compensation structures in the masking layer can be used, but require complex fine-tuning of the mask design and exact knowledge of the orientation dependence of etching rates.

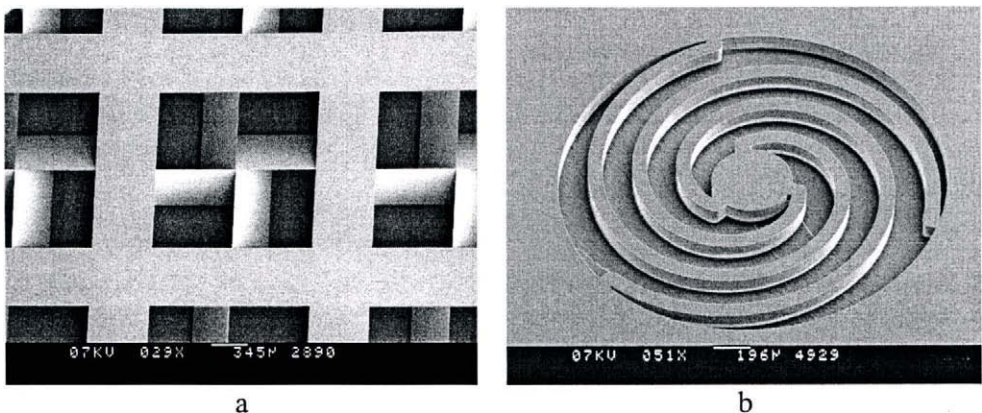a   b

Fig. 2.   a) Rosettes etched in $\{100\}$ silicon in 1, double-sided KOH etch step. b) Free supported valve spring structure etched in one wafer by reactive-ion etching, followed by wall-passivation and isotropical wet-chemically underetching in $\{111\}$ silicon.

The surface roughness of anisotropically etched channels can be high, depending on the exact conditions of etching (temperature and composition of the etchant). Recent work has shown that this roughness is inherent to anisotropic etching: e.g. on {111} planes etching only proceeds when nucleation of etch pits is initiated by defects (e.g. oxygen-induced stacking faults) [19].

If smooth surfaces are required, isotropic wet-chemical etching is the preferred method. Since this etching process is not limited by surface reactions, as is the case in the anisotropic method, but by mass transport in the solution, under optimised conditions, any protrusion from a nominally flat surface will be removed at a higher rate than that flat surface, and features are rounded off, while surfaces become smooth. The channel shapes obtained with this method are tubular. Fig.3 shows a typical result. Design freedom is limited with this method as well, since the width of the channel will always be at least twice its depth due to underetching under the mask.
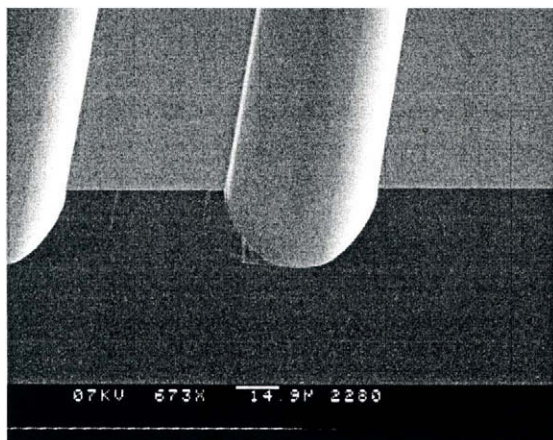

Fig. 3.   Cross-section of isotropically etched channels in silicon; note that the width of the channel is almost twice its depth.

Anodic wet-chemical etching exploits an anodic potential applied to the substrate to induce etching, and gives essentially the same results as the previous method. However, if silicon is etched in HF-solutions at low anodic current densities, it transforms into a porous layer, of which the pore size distribution is well-defined and can be controlled accurately [20]. It was demonstrated that, by variation of the current density, freestanding structures of porous Si can be fabricated, as shown in Fig.4 [21], with a pore size of ca. 13 nm. Many applications of such a porous material in micro biochemical analysis and micro reactor devices can be imagined. Some interesting applications have already been realised. For example, it was reported that porous Si, used in an enzyme reactor, shows a 100-fold increased enzyme activity, compared to a non-porous reference [22]. Porous silicon can be modified in many ways, e.g. it can be

oxidized to obtain porous silicon oxide [23]. In this way many different materials with a high surface-to-volume ratio can be manufactured (if desired, in a tubular channel form like in Fig.4), which can be used in gas chromatography for fabricating molecular sieves (microdialysis), for gas sensors or as very effective catalysts in heterogeneous gas phase microreactors. Structures like in Fig.3 are very interesting for the latter application, since it is possible to micromachine a construction in which gases can be passed from the central to the next channel, while (although this requires quite complex processing) the outer channel in Fig.4 could be used to pass a cooling substance.
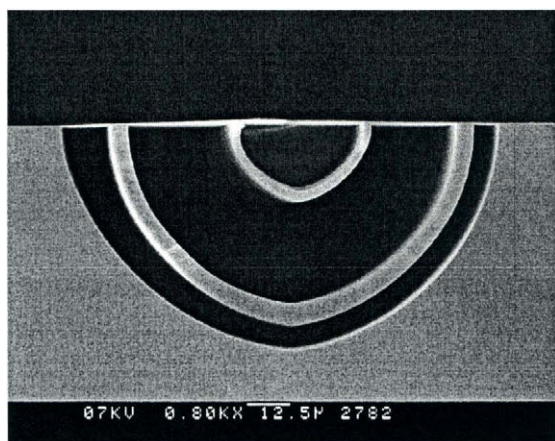

Fig. 4.   Several concentric channels composed of nano-porous silicon, attached to a silicon nitride mask
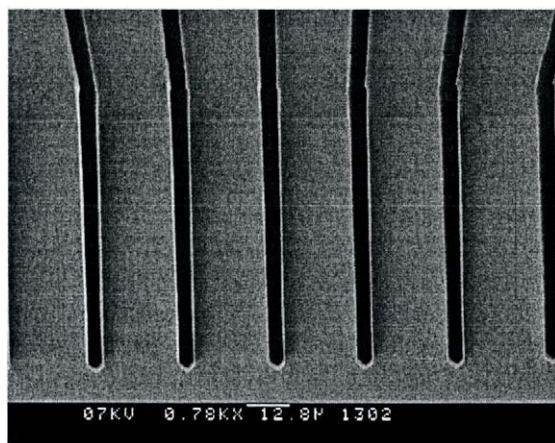

Fig. 5.   Deep trenches etched in an SF_6-O_2 plasma; the walls of the ca. 4μm wide trenches are covered with a silicon nitride layer.

Fig.5. gives a typical result of channels fabricated with Reactive Ion Etching (RIE), in this case in an SF_6-O_2-based inductively coupled plasma process. This method allows the largest degree of freedom of structural design: the pattern defined in the mask can directly be projected into the silicon substrate, allowing sharp corners and channels with in-plane tapers [24] an high depth/width aspect ratios (feasible: 10 for all etch shapes and up to 20 for trenches). The number of channels per unit area can be as high as the lithographic steps allow; state-of-the-art optical lithography achieves feature sizes of less than 0.25 μm, while with laser interference methods repetitive features smaller than 100 nm can be achieved [25].

2.2.3 Sacrificial layer etching

The previously described methods are based on the fabrication of structures into a silicon substrate (bulk micromachining). Another approach is to build structures *on top of* the substrate surface. The latter method is called surface micromachining, and has become famous due to the tiny micromotors that have been presented in many different configurations throughout the literature [26]. The fabrication procedure consists of stacking and patterning layers of thin films, and subsequently selectively removing of the layers in between (the sacrificial layers), therewith creating gaps in-between the etch-resistant layers. The method is not limited to the conventionally used combination of polycrystalline silicon as a structural layer and silicon dioxide or doped silicate glasses as sacrificial layers, but can be applied to any combination of materials that can be etched selectively with respect to one another. In fact, the first demonstration of the method was performed by Nathanson et al. in 1967, who used a combination of gold and nickel films to fabricate a so-called "resonant gate transistor" [27].

A notorious problem in surface micromachining is stiction: in the drying step after sacrificial layer etching, the freestanding thin layer structures may stick to the surface underneath as a result of capillary forces during withdrawal of the liquid meniscus under these structures. This is an example of the scaling laws in miniaturization: surface effects become more prominent when feature size goes down. Freeze-drying is the most commonly used solution for the stiction problem. In section 3.7, an example of a surface micromachined structure will be given, viz. a nanofluidic bubble pump.

2.2.4 Sacrificial molding in silicon

Although the applications of silicon channels are numerous, their use is limited in some of the current fields of interest in chemical analysis. For example, in Capillary Electrophoresis (CE) high electrical fields along the length of a channel are required, which is not possible in silicon because of its high electrical conductivity. Another serious drawback of silicon is that it

exhibits no optical transparency for the visible or UV wavelength regime, frequently used for detection. For such applications, a more suitable material like glass is highly desirable.

Bulk micromachining of glass will be described in the next section, but another particularly interesting strategy is to use silicon channels as a mould for the deposition of insulating materials, like silicon nitride or silicon dioxide. This turns out to be very feasible [28,29]. Fig.6 shows some examples, in which a combination of silicon etching, silicon dioxide deposition, and anodic bonding to a glass plate was applied, resulting in micro Transparent Insulating Channels (μTICs). Earlier results have shown that these channels support electrical field strengths of up to 4 kV/cm and are well-suited for CE [29].
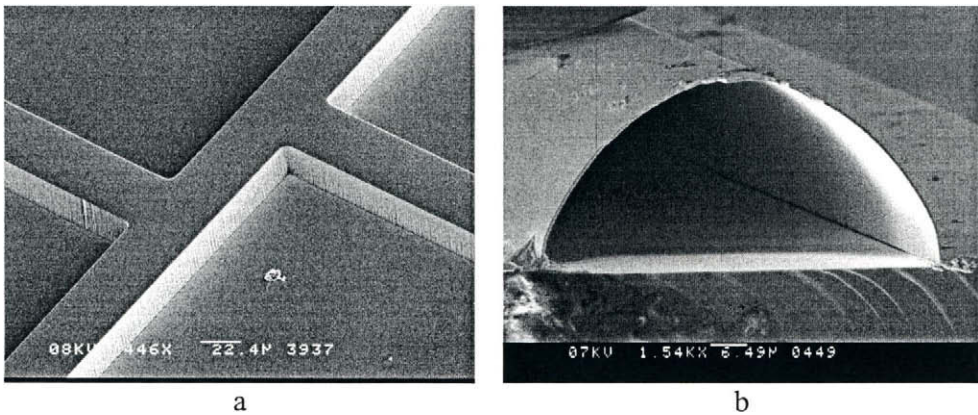

a   b

Fig. 6.   Examples of "μTICs". The left channel is based on a channel etched in Si by RIE, the right channel results is a replica of an isotropically etched Si pattern
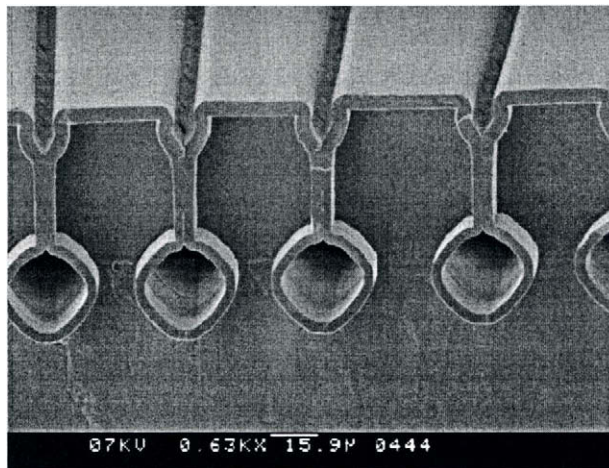

Fig. 7.   Silicon nitride tubes, buried underneath the surface of a silicon substrate

Since these channels may have very thin walls and small diameters, completely new types of fluidic devices become feasible. An additional important advantage of the use of glass-like materials is that they are transparent for light in an interesting wavelength regime for optical detection of e.g. biochemical components. It is also possible to fabricate channels at different depths below the surface of a silicon substrate [30], thus leaving the silicon substrate surface free for the implementation of other microstructures or electronic circuitry. Such channel structures, fabricated by a combination of RIE, isotropic wet-chemical etching, and reactive sealing with a Low-Pressure Chemical Vapour Deposition (LPCVD) silicon-rich silicon nitride coating, are shown in Fig.7.

2.3. Wafer bonding

To realize closed structures for fluidic applications, bonding of (patterned) substrates is often used. For this purpose, several methods are available. An excellent comprehensive review of wafer bonding processes and the related fundamental principles can be found in a recent book by Gösele en Tong [31]. Here we give a short overview of the most commonly used methods. These are:

1. Direct bonding (without intermediate layer, also called fusion bonding); this process can be used to bond silicon to silicon, which leads to a monolithic structure, with the advantage of reduced stresses when the structure has to undergo temperature cycles. The bond can already be relatively strong at room temperature, however, excellent and durable bonding is obtained after annealing at high temperatures, e.g. at 1000°C. The most sophisticated example till today is a package consisting of 6 individually etched silicon wafers [32]. An excellent overview is given by Plößl and Kräuter [33]
2. Anodic bonding (also called electrostatic bonding), mostly of a combination of silicon and Pyrex glass substrates. This method consists in applying a high electrical field across the wafer sandwich, at a temperature close to 450°C [34]. Due to ion diffusion in the glass at these conditions, a space charge region forms at the surface of the glass wafers, which leads to a strong electrostatic attractive force between the two wafers. Thus, the gap between the wafers is closed, and oxidation takes place at the interface, leading to a tight bond. The method also works with a thin glass layer in-between two silicon wafers [35].

The demands on surface roughness are low for anodic bonding, asperities up to 1μm are allowed. Direct bonding however requires a very smooth surface, with roughness below 1nm. This can normally only be achieved with special polishing techniques [36].

2.4. Glass micromachining

For many applications in solution chemistry, for either synthesis or analysis, glass may be the preferred material, due to the familiarity of the chemist with the material, chemical resistance to many solvents and optical transparency. In order to obtain the desired microstructures in glass, batch fabrication techniques similar to those used for silicon micromachining, based on photolithography, may be used. After the pattern is transferred lithographically into a resistant coating (a polymer layer or a metal), etching of glass is performed in solutions containing hydrogen fluoride (HF). With this process, it is possible to achieve features with a size down to several tens of nanometers. As was already noted for the isotropic etching of silicon, the width of any structure will be at least twice its width, such that deep trenches are not possible. Glass and fused silica removal rates are typically in the order of 0.1 to 1 μm/min. The method is therefore particularly useful for shallow structures. Its main drawbacks are the extreme health hazards involved with HF and the clean room facilities required for the photolithographic process, leading to relatively high fabrication costs.
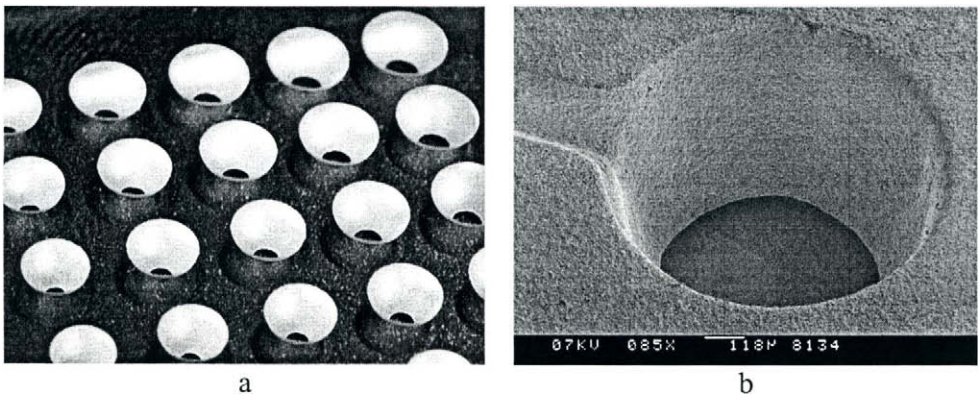

Fig. 8.   a) Through-holes in 1.1 mm thick borofloat glass, blasted with alumina oxide particles and masked with a polymer film, b) close-up of a hole with channel, blasted in an alumina oxide substrate with silicon carbide particles. The copper mask is still on in figure b.

A cheaper and less critical process to obtain microstructures in glass was developed in the last decade. This so-called "powder blasting" process, developed for e.g. flat panel displays [37] and micro counter flow heat exchangers [38], was recently exploited to fabricate fluidic structures for capillary electrophoresis [39]. Glass removal rates with this abrasive process, which consists of directing a jet of powder particles onto a brittle material in order to achieve local erosion, are typically between a few μm/min and 1mm/min. Thick photo-elastomeric coatings that can be photolithographically patterned are very suitable masks for this process, and structural features with a

size of $50\mu m$ and larger can be reliably manufactured. These dimensions are in the range of interest for the fabrication of chromatographic channels and connection holes. Fig.8 shows typical examples of structures obtained with this method. Picture 8b demonstrates that the powder blasting method is not only applicable to process substrate materials like glass and silicon but also to make channels and holes in ceramic materials like aluminum oxide. Next to elastomer mask materials, also thick deposited metal layers were used to increase the erosion selectivity between mask and substrate material. Besides the benefit of the erosion rate, powder blasting requires less stringent safety precautions and a less expensive infrastructure, so that this method may be a cost-effective alternative to HF etching. The main drawback of powder blasted with respect to HF-etched fluidic channels is the much higher roughness of the surface of the channels, which generally are a few μm and below 50nm for powder blasting and HF-etching, respectively.

3. EXAMPLES OF FUNCTIONAL DEMONSTRATORS MICROMACHINED IN SILICON AND GLASS

3.1. Introduction

Although silicon may generally not be the material of first choice for applications in the field of fluidics for (bio)chemistry and medicine, the extended toolbox of fabrication methods makes it a very attractive material for the demonstration of microfluidic principles for these fields. In particular, the mono-crystalline nature of silicon substrates in combination with anisotropic etching processes, the precise dry-etching techniques and sophisticated thin-film surface micromachining processes, can be used to create a large variety of innovative microstructures. Several examples of such micro- and nanofluidics structures for (bio)chemical and medical analysis will be presented and discussed.

For many chemical, optical and high-voltage applications, glass is the best solution. Therefore a few examples of applications will be given in which glass is used, combined with silicon (transparent top layer) or in a full glass-glass sandwich. This section will finish with examples of devices made with surface micromachining technologies, to provide e.g. further size reduction.

3.2. Hydrodynamic chromatography chip

A first example of a device for which very precise micromachining in silicon is essential for the device's functionality, is the so-called hydrodynamic chromatography (HDC) chip. In this device, essentially consisting of an 8cm long, 1mm or 0.5mm wide and $1\mu m$ high separation channel, small particles and

large molecules are separated in a parabolic (pressure driven) flow profile [40]. The hydrodynamic chromatographic separation in such a micromachined channel is based on the principle that in narrow conduits (effective size $< 1\mu m$) with laminarly flowing liquid (Fig. 9) larger molecules or particles (sizes ranging from 0.002 to 0.2 of the conduit size) are transported faster than smaller ones, as they cannot fully access the low-velocity regions near the conduit walls [40, 41]. In HDC this is used for analytical separation of biopolymers or synthetic polymers in similar applications as in traditional Size Exclusion Chromatography (SEC). Unlike the latter, HDC has no stationary phase and is therefore faster and more efficient. However, part of its performance is lost by imperfect instrumentation when performed classically in packed columns [42] or open capillaries [40]. The former suffers from extra-column peak broadening, the latter from too low detection volumes.
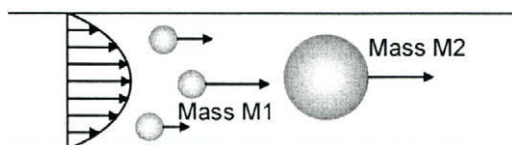

Fig. 9.   Principle of HDC separation. Larger analytes cannot sample low fluid velocities near the channel wall and therefore move faster
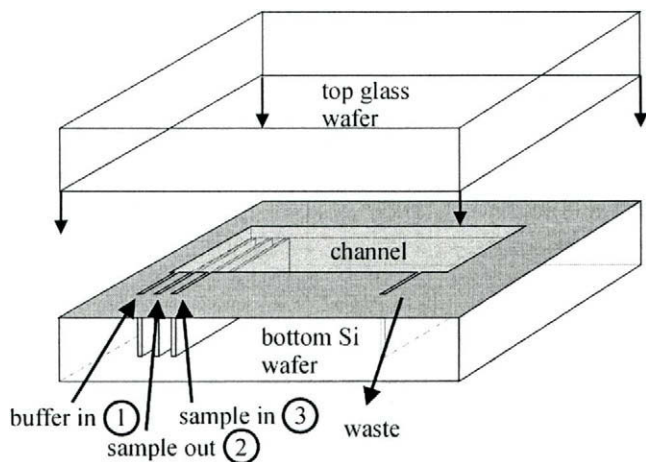

Fig. 10.   A planar HDC-chip. The injection slits are shown on the left

An integrated on-chip HDC system (Fig. 10) should both preserve the efficiency and provide more material for detection by using a shallow but wide separation channel. Other advantages are negligible solvent and sample consumption and easier temperature control as a thin channel exchanges heat much easier than robust columns.

For a good quality of the separation, a very small variation in the channel height is mandatory. With the aid of sacrificial layer techniques, a variation of channel depth of less than 0.5% across a 0.5 mm wide channel was realized. Furthermore, with a state-of-the-art deep reactive ion etching method, it was possible to machine very narrow slits in the silicon substrate that is bonded to a glass substrate to enclose the liquid channel [43]. Three of these slits serve as an integrated injector, which ensures the introduction of a very narrow plug of analyte in the separation channel (Fig. 11). The precise control of separation channel height and injector geometry resulted in the successful separation of a mixture of polystyrene particles of 26, 44, 110 and 180nm diameter in less than 3 minutes [44]. An example of such a separation is shown in Fig. 12 and 13. Additionally, the separation performance was demonstrated by separation of large biomolecules and of several small molecules. In the latter case the separation mechanism is not HDC but interaction with the relatively large channel area.
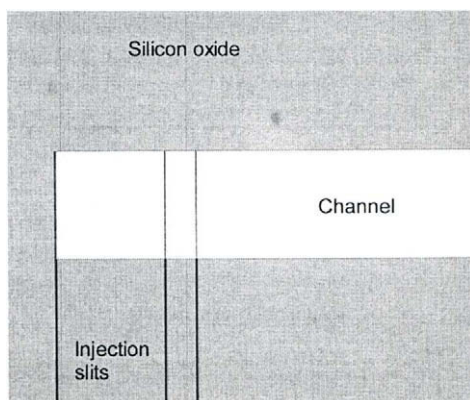

Fig. 11. Top view of injection slits and separation channel.
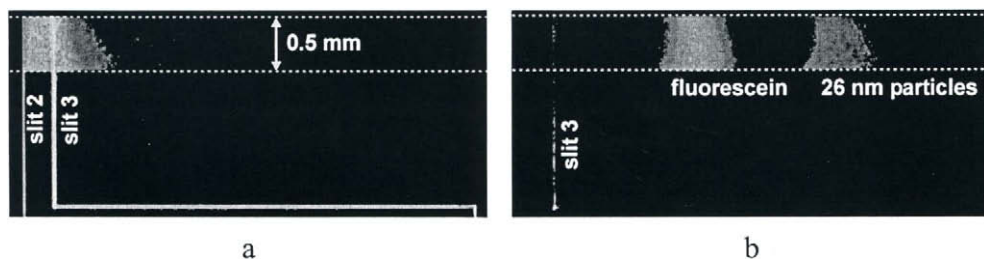

a   b

Fig. 12. HDC separation of 26nm particles from fluorescein: a) injection of the sample plug, b) separation of the different fractions

Further optimization of the channel uniformity and the resulting separation performance was achieved by fabricating the chip completely out of fused silica, again using reactive ion etching to define the injection slits as well as separation channels. Fusion bonding was used to connect the top and bottom wafers (reduced deformation compared to silicon-glass anodic bonding).
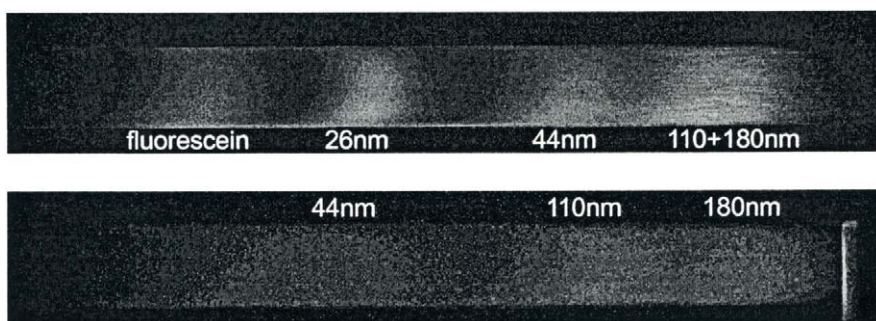

Fig. 13. Separation of various polystyrene particles within 3 minutes in a top view of a 0.5 mm wide separation channel, taken at 30 mm (top) and 79 mm (bottom) from the injection structure. In the bottom picture the deeper waste channel is visible on the right.

3.3. Optical absorbance detection cell for FIA system

In a second example, the crystalline nature of silicon is exploited to create an optical absorbance cell with a long (10mm) path length. Such a long path length makes it possible to obtain the required detection limit for sensing the reaction product, indophenol blue, which is formed during the analysis of ammonia samples in a miniaturized Flow Injection Analysis (FIA) system. The complete system is discussed in the next section. In order to reflect a vertically oriented light beam into the horizontal flow channel, a 45° reflecting sidewall is needed, which can be made using silicon {110} planes, that are exposed on a patterned silicon crystal after etching in a KOH-IPA solution. In Fig.14 and 15 the schematics of the set-up and the realized detection cell are shown. The realized cell was found to give the desired detection limit for ammonia of 0.1mM. More details can be found in [45].
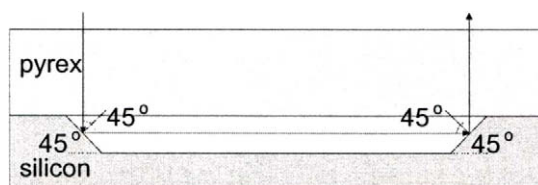

Fig. 14. Principle of the optical absorbance chip realized in silicon/glass: the optical path length is increased by reflecting the light beam under 45 degree angles
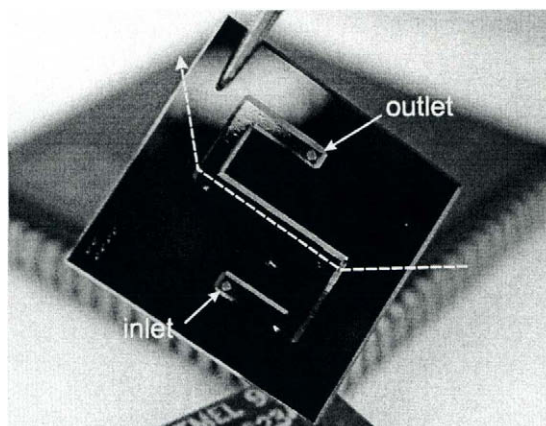
Fig. 15. In a silicon-glass sandwich processed light absorbance detection cell. The dashed line shows the light path.

3.4. Rapid vortex micromixer

A nice demonstration of the application of deep reactive ion etching is the development of a high-speed liquid mixer. With RIE a large number of tangentially entering entries into a cylindrical mixing chamber (Fig.16 and 17) [46] was etched into silicon. High mixing speeds are obtained due to the lamination of the alternating inlets of the two liquids to be mixed (Fig 16b and 18). In order to get a good uniformity of the mixing, precise geometric control of the inlet channels is required. Fig.17 shows the realized silicon micromixer chip. Simulations of the device have indicated that indeed sub-millisecond mixing should be possible (Fig.16b).
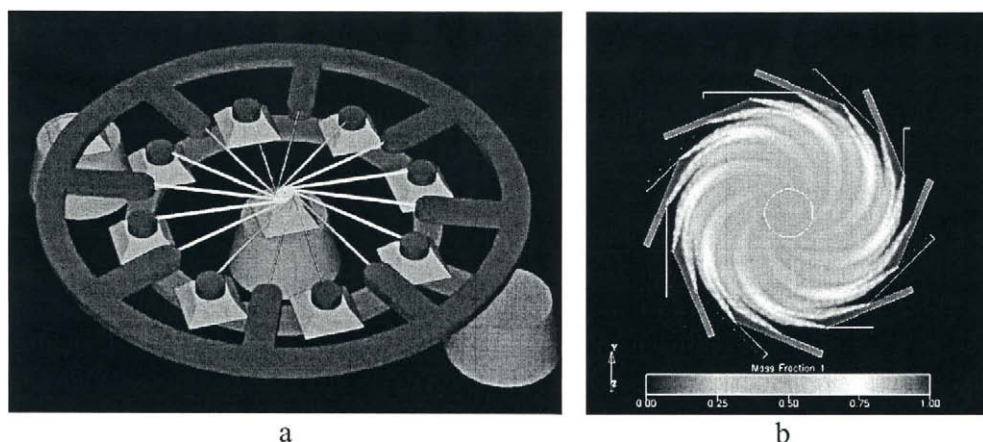

a   b

Fig. 16. Design (a) and simulation (b) of an ultra fast tangential mixer chip in a glass-silicon-glass sandwich. Simulations were performed by Coventor.
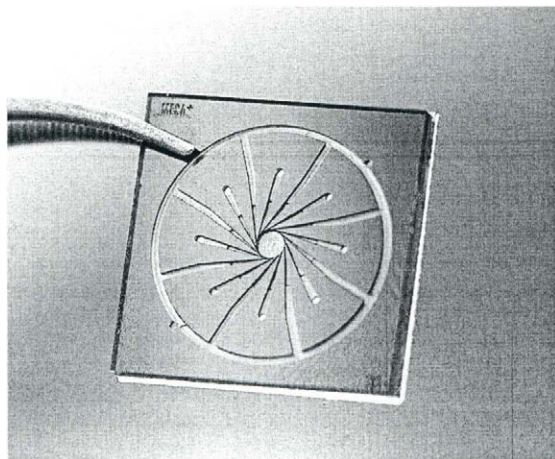
Fig. 17. Realized ultra fast tangential mixer chip in silicon with glass cover plates at both sides: the outer ring transports chemical one, whereas the inner ring, processed in glass at the backside transports the other compound.
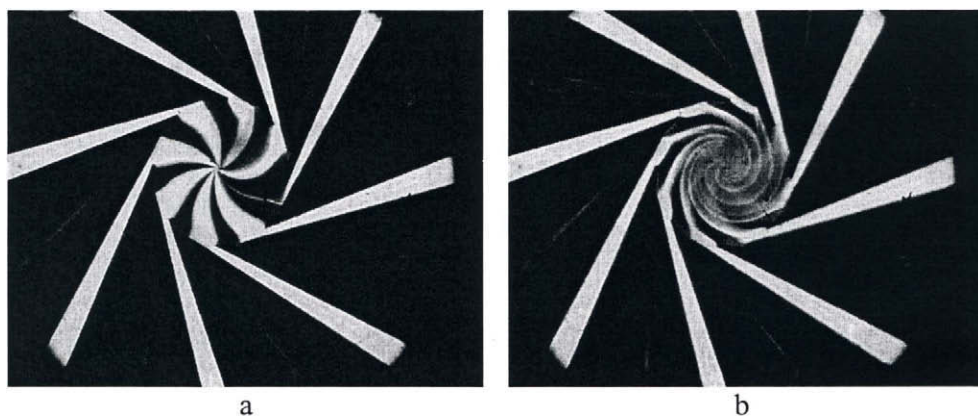

a   b

Fig. 18. Experimental results of the mixing of clean water and water with fluorescein at a) 0.1ml/min. and b) 1.0ml/min.

3.5. Silicon micromachined hollow microneedles

Nowadays, typical routes for drug delivery are either through hypodermic needles, via (iontophoretic) patches or by oral or respiratory administration, while diagnostic sampling in most cases requires extraction of blood through a hypodermic syringe needle, followed by analysis of blood components in a specialized laboratory environment. These methods all have some disadvantages, depending on the particular drug that is to be administered, or the analysis that is to be performed. For example, in some cases the methods are too slow, not effective, or wasteful with respect to the volumes of blood or other

substances that are consumed, or cause too much inconvenience for the patient, like pain and skin irritation or damage.

During the last decades, it has become clear that the introduction of MEMS offers exciting opportunities to advance the medical field, because of the ever-shrinking size of the resulting devices. This allows minimally invasive methods, causing limited tissue damage and pain reduction. In addition, miniaturization of analysis methods enables the development of versatile portable equipment for "point-of-care" monitoring and treatment of patients. Ultimately, the advancement in the field may reach the state of drug-on-demand possibilities, by which it is meant that monitoring and dispensing components are integrated in an intelligent feedback system, so small that it can be carried on the body of the patient, while it is continuously connected to the blood stream and releases the required drug whenever a certain monitor analyte reaches a critical value.

Although the current situation is still far away from that stage, one step in the right direction is the development of arrays of micromachined hollow microneedles for transdermal liquid transfer. Such hollow needles can be used for blood extraction, drug delivery, or both. The basic idea behind the microneedle approach is that due to the small size of the needles, the penetration of the skin will only be shallow such that tissue damage will be limited and pain sensation will be avoided. The necessary total fluid flow will be achieved by using a high-density matrix of needles.

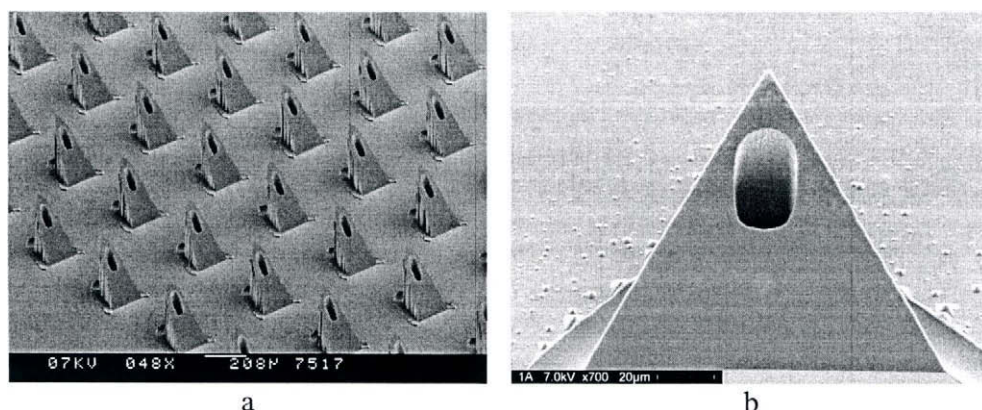a   b

Fig. 19. Matrix of hollow silicon micro needles (a) and one micro needle in detail (b).

Several approaches to the micromachining of hollow microneedles for transdermal application are known, and roughly, these can be divided in out-of-plane and in-plane designs, the plane in this case being the surface of a thin (silicon) plate, which contains an array of microneedles. One of the possibilities for creating out-of-plane hollow micro needles with the proper mechanical strength and sharpness to be applied for painless transdermal transfer of liquids is discussed in detail in reference [47]. The fabrication method allows different

needle shapes like blades and pencils and is robust enough to be applied for larger-scale production. Essential features of the design are that the location of the opening for liquid transfer does not coincide with the needle tip and can actually be positioned freely, a flow channel extending to the opposite side of the substrate leaving enough space for any desired fluidic component on that side, a high needle density, and a needle structure with knife-like cutting properties.

Briefly, the fabrication method consists of a sequence of deep Reactive Ion Etching, anisotropic wet etching and conformal thin film deposition steps on {100} silicon wafers. The crystallographic nature of the silicon substrate is utilized for the fabrication of sharp edge. Using a combination of anisotropic etching in aqueous KOH and deep RIE it was possible to fabricate a large matrix of such sharp needles (Fig.19). Besides the optimized mask design, also the appearance of a {111} silicon crystal plane on the sidewall of the needle adds to the sharpness.

3.6. Full-glass chips for capillary electrophoresis and microreactors

At the MESA$^+$ labs and commercialized by Micronit Microfluidics B.V., glass etching, powder blasting and glass-glass direct wafer bonding are standard technologies nowadays. With this portfolio on techniques, full-glass chips can be made which are very suitable for applications using on-chip chemistry, capillary electrophoresis, electro-osmotic flow control and optical detection.

On-chip capillary electrophoresis (CE) is a well-established analysis technique. The requirements are: long (few centimeters), narrow (few ten micro meters) and shallow channels (up to 20 micrometers), processed in a good insulating substrate material like glass. Next to a good electrical insulation, glass is also beneficial for its optical transparency, which allows optical detection like for detecting fluorescently labeled DNA. Figure 20 shows typical examples of long CE chips for optical detection and with integrated electrodes for conductivity detection of ions.

Since the walls of glass chips are charged, electro-osmotic flow (EOF) can be generated when applying an electric field over the channel length. This EOF can be used to drive flows in microreactors. Figure 21a shows a picture of such a system with integrated electrodes.

After the chips are etched, holes are powder blasted and the layers are fusion bonded, electrodes can be deposited (sputtered or evaporated) with little effort through a shadow mask (usually a thin, laser-cut stainless steel plate or etched silicon membrane). Since the shape of powder blasted holes is not cylindrical but conical, there is perfect step coverage of the deposited layer such that excellent electrical contact exists between the electrode area at the top layer of the chip and the walls and bottom of the blasted hole. Therefore these deposited electrodes can be used to establish electrical contact from the outside

to the inside of the channel in combination with e.g. glued fiber connections, o-rings or Nanoport connectors [48]. Next to electro-osmotically driven reactors, pressure-driven reactor chips were made such as the one shown in figure 21b, designed for 360µm od. / 40µm id. silica fiber capillaries.

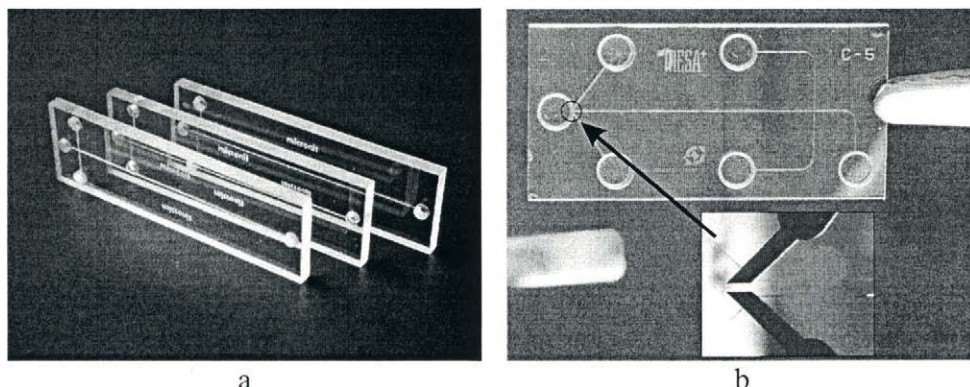a   b

Fig. 20. Glass chips for e.g. capillary electrophoresis separations (a), figure b) shows a CE chip with integrated electrodes for conductivity measurements (see close-up) to detect ion concentrations.
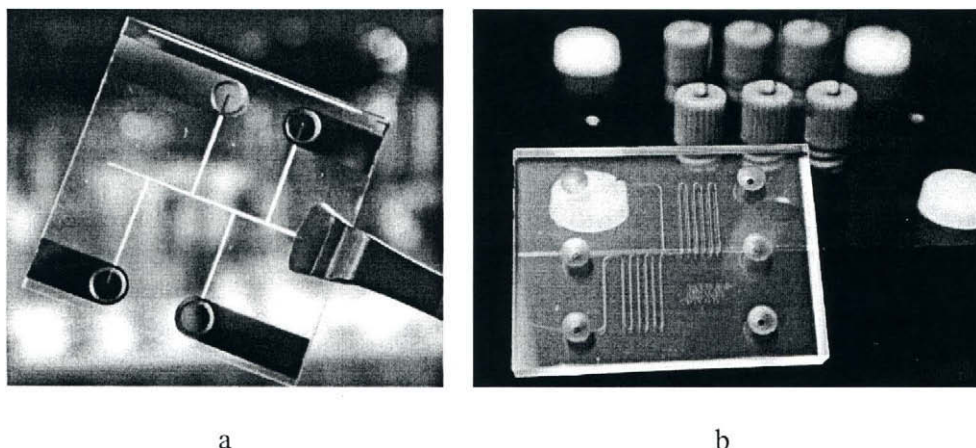

a   b

Fig. 21. a) Reactor chip with integrated electrodes to drive electro-osmotic flows, b) pressure-driven reactor chip with Nanoport connectors for 360µm od. / 40µm id. silica capillary fibers.

3.7. Sacrificial layer technology for nanofluidics

Sacrificial layer technology is a very promising direction to further downscale the dimensions in one or two directions, to get below 1µm. Applying the technology to channels allows us to enter the area of nanofluidics. This is the domain where surface effects like wall charges (double layers) and surface

tension start to dominate liquid behavior, while ultimately a liquid can no longer be considered as a continuum but as individual molecules. Bulk behavior of the liquid, such as fluid flow described by the differential equations of Navier-Stokes with zero boundary slip at the walls and macro descriptions about dissolved molecules and ions, are no longer valid. New phenomena that could be used for separating and detecting species appear.

A very versatile technique to fabricate channels with nanometer dimensions (in the vertical direction) is a combination of sacrificial layer techniques and bulk micromachining [49]. Using this technique it was possible to make channel crossings, flow sensors, and perforated membranes. If these and other components are combined on one chip, very small, integrated fluidic systems can be made. As an example of the potential of this concept we realized a pneumatically actuated, capillary / pressure driven micropump, capable of delivering picoliter amounts of liquid in a controlled way (Fig.22). Optimal exploitation of the large role of surface effects will make the realization of complex micro- and nanofluidic networks possible with interesting applications such as fast fluidic computing and ultra low volume high throughput experimentation and analysis. Full details can be found in [40].
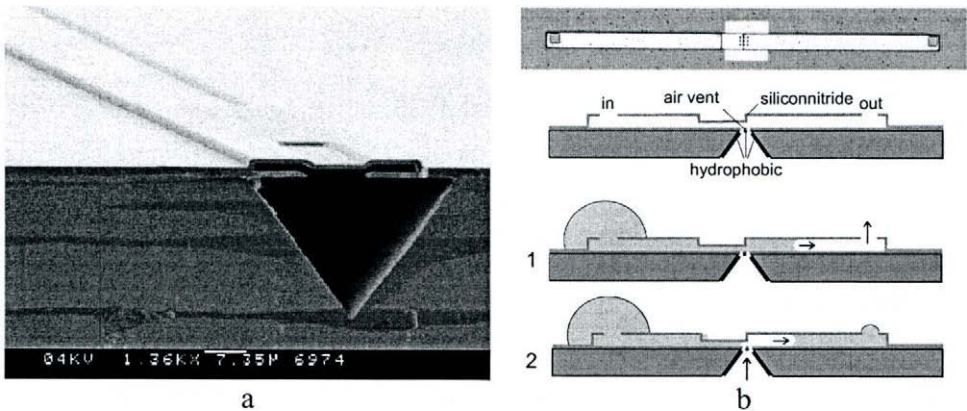

Fig. 22. a) Illustration of structure made with a combined bulk / sacrificial layer etching technique. b) Applied to a picoliter bubblepump: due to the asymmetric shaped channel, liquid will be pushed out by air pressure in one preferred direction (step 2). Refilling with liquid takes place by capillary forces (step 1). The upper picture shows the top view of the system.

4. INTEGRATION

One of the opportunities that are offered by microsystem technology is the possibility of advanced integration of functional modules. When all functional elements are processed by subsequent fabrication steps at wafer-cale, it is called "monolithic integration". The alternative is hybrid integration; composing of a system by individual chips afterwards (chip-scale). Reasons to aim at hybrid or monolithic integration of functional elements are:

1. Reduction of signal quality loss. This is obvious for modern day microelectronics: the weak electronic signals will never reach their final destination in a good shape if long metal wires with large parasitic capacitances, resistances en noise pick-up are used. In fluidic systems consisting of microchannels, resistances for flow can be high, and will require considerable pressure to be maintained; capacitance may exist in (flexible) tubing or dead volumes in fluidic connections, leading to the "loss" (i.e. dispersion) of fluidic signals (i.e. sample plugs). For such applications, monolithic integration may be beneficial, of even crucial. Similarly, for reasons of heat management, or protection against the environment, integration may be advantageous.
2. Delay time reduction. For example, after reactions have taken place, the reaction product needs to be analyzed. The longer the delay time between reaction and analysis, the further the reaction products might degrade. So for analysis without the possibility to quench the reactions or to stop the degradation of the product (e.g. kinetics studies), direct analysis of the product is required, which can be achieved by functional integration of the sensor on the reaction chip.
3. Advanced miniaturization. This needs no further debate, all the advantages of miniaturization have been extensively discussed in this book.
4. Similarity of components. For applications in high-throughput screening, a large amount of similar components operating in parallel on the same substrate may be preferred for reasons of dead volume, reduced footprint, and ease of fabrication. In this particular case, monolithic integration may offer the advantage of minimized variation in component properties.

Continuous measurement of the ammonia concentration in environmental water samples was chosen to study integration concepts. The classical colorimetric method for ammonia determination based on Berthelot's reaction, in which several reagents have to be added sequentially in specified portions, was taken as a starting point for the development of a miniaturized flow injection analysis (FIA) system [51]. Miniaturization was used to allow to develop portable systems with reduced weight, size, energy consumption, reduced

consumption of reagents and production of waste, faster analysis due to smaller dead volumes and shorter diffusion path lengths, improved control of liquid flows and faster heat exchange. With typical dimensions of 10 to 200μm wide channels, the species transfer is in the Aris-Taylor regime, where radial diffusion is fast compared with residence time, which results in reduced band broadening and detector signals that are symmetric (Gaussian) as a function of time, in contrast with detector signals in conventional FIA systems.

The system layout is shown in Figure 23. A sample plug is fed from a side branch into a main channel with continuous flow of de-mineralized water carrier. While this sample plug travels through the system, the first reagent, hypochlorite in a pH 11 buffer solution, is added. To ensure complete reaction, the sample and the reagent are mixed, and further down the main channel, the second reagent (phenol and the catalyst nitroprusside) is added and mixed as well. After the second mixer, the temperature is controlled at 37°C in a reactor channel and kept at elevated constant temperature for a specified time. Next, the mixture passes the detection cell described in section 3.3, in which light absorption at a wavelength of 590 nm is measured. From this absorption measurement, the original amount of ammonia is calculated.
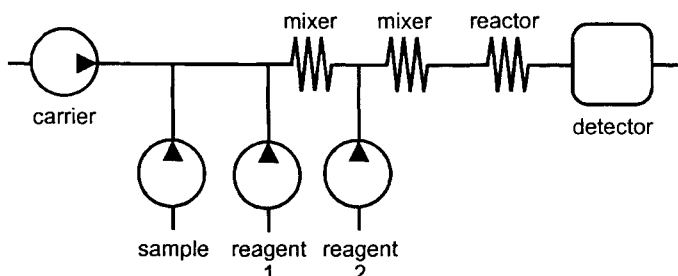

Fig. 23. System layout of an analysis system for the detection of ammonia

Three main concepts for connecting microfluidic components are known in literature. The first approach is vertically stacking of components [52], which was done in order to minimize the length of the connections between the various components. The second approach is planar monolithic integration of components [15], which, similar to monolithic VLSI of microelectronic components, leads to the most advanced degree of miniaturization. The main advantage of this approach for fluidic circuits is that dead volumes and fluidic paths are reduced to a minimum. An often-cited example of an early micro fluidic analysis system is the gas chromatograph with integrated injector and detector on a 5 cm silicon substrate published in 1979 [2].

The most important drawback of monolithic integration is that after failure of one component the complete system might be useless. To avoid this, either sufficient backup functionality needs to be incorporated in the monolithic system to take-over the functions of the malfunctioning elements, or the system

needs to be disposable. The latter approach requires very high market volumes and rather simple, high-yield processing to reduce the costs.

For systems that do not meet these conditions, a hybrid solution is preferred. One way is by a concept of "Modular Assembly Total Analysis System", MATAS [53]. The microfluidic components are packaged in a housing to form a robust module that can be soldered into a Printed Circuit Board, PCB: a base plate with on one side all necessary electronic circuitry to control and read out the system, and on the other side a channel plate with fluidics connections for the components. Next to the benefit of replacing malfunctioning components, the hybrid system offers also the possibility to change the functionality of the system in order to perform e.g. a different chemical analysis. For example, the reaction chamber could be replaced with one that is designed for a different temperature range. Also, the sensor element can easily be replaced by a different type of sensor, e.g. a conductivity sensor.

Peristaltic pumps, capable of bi-directional pumping and self-priming, were realized. Each consists of three pump chambers in a row, fabricated with RIE and through-holes that serve as in- and outlets, created by powder blasting. Each pump chamber has a deflectable glass membrane realized by using selective anodic bonding [54] and subsequently thinning down (HF-etching) of a borosilicate plate. The membranes are actuated by glued piezo-discs. Measurements showed a maximum flow rate of 30 μl/min.

To ensure mixing within seconds in the laminar regime, a simple mixer was designed that consisted of a Y-junction, which runs into a narrow channel. [55]. The mixers were realized in silicon using RIE. The actual conversion of ammonia into indophenol is performed in a reaction coil, in which the temperature of the passing reacting mixture is 37$\pm$0.5°C for 30 seconds (RIE in silicon). Temperatures in the reaction chamber are controlled by platinum heaters and temperature sensors.

For absorbance measurements, the detection cell, described in section 3.3 was coated with high reflectance coating (ca. 40 nm of Ag or Pt is optimal for a wavelength of 590 nm).

For both, the hybrid and the monolithic integrated systems the same component designs were used with the difference that for the modular hybrid system all components were designed as separate stand-alone parts, whereas the components for the monolithic system were designed in one wafer with connecting channels. The fluidics plate of the hybrid system was built out of DRIE etched channels and powder blasted connection holes in a silicon wafer, which was anodically bonded to glass. A photograph and a schematic drawing of the completed hybrid system are given in Fig.24.
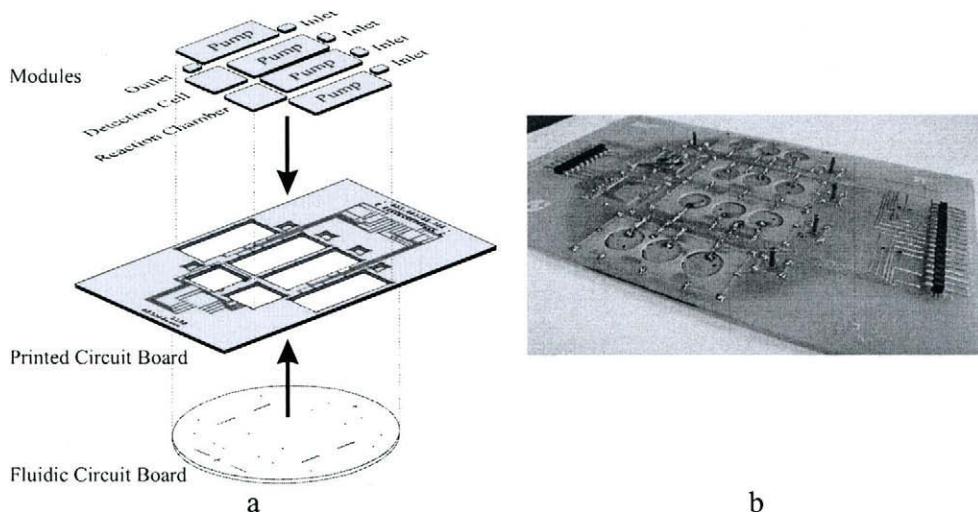
Fig. 24. Modular hybrid analysis system according to MATAS approach, a) schematic drawing (exploded view), b) photograph of the manufactured system with mounted modules.

Essential for monolithic integration is that all components can be realized from the same materials and in one fabrication process, such that the designs must be compatible from a technology point of view. The monolithic analysis system was realized in a bonded pair of a silicon bottom wafer and a borosilicate glass top wafer, each with a diameter of 10 cm. In the silicon wafer a channel structure was etched with DRIE which connects the various components of the system. After deposition of a thin SiO_2 layer to electrically insulate the silicon, metal lines of chromium and platinum were deposited, which are needed for the driving the piezo actuators of the pumps, as well as for heating the reaction chamber and monitoring the temperature of the solution. In the top glass wafer, cavities where etched with 50% HF to thin-down the glass locally in order to realize thin pump membranes. A photograph and a schematic drawing of the monolithic system are shown in Fig.25. A more detailed description of the fabrication process of the monolithic integrated ammonia analysis system can be found in [56].

Both ammonia analysis systems were tested and the performance was according to specifications. The modular hybrid system could detect ammonia concentrations ranging from 5.6mM to 560mM. These specifications are comparable to the performance of commercial available desk-top systems. The monolithic integrated system can be further optimized reducing the dead volumes of especially the connections between the components.
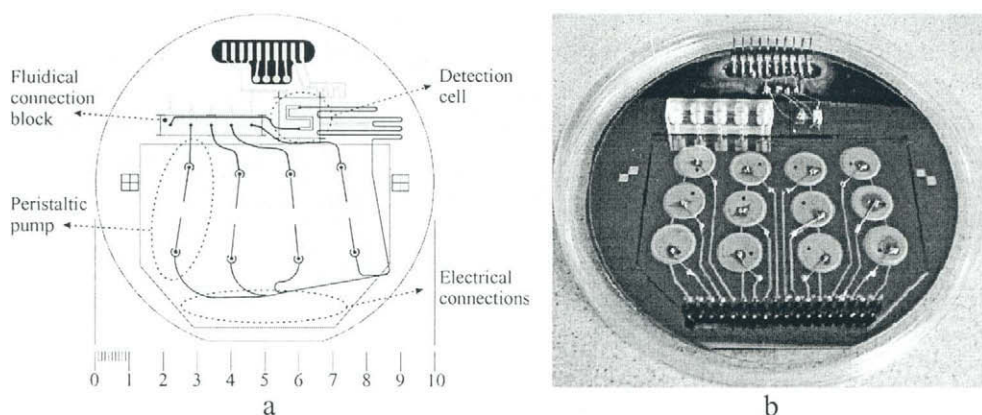
Fig. 25. Monolithic integrated analysis system: a) schematic drawing, b) photograph of manufactured system with a diameter of 10 cm.

5. CONCLUSION

In this article, a number of demonstrators was discussed, fabricated with the aid of silicon and glass micromachining technology. Although at the end of the day silicon may not be the material of choice for a commercial product, it certainly has its value for the proof-of-concept stage in a development process. In particular, for future scaling down of fluidic structures, silicon microtechnology is much more advanced than any technology to machine other materials at this moment.

Glass on the other hand is very well known in today's chemical labs, such that the threshold to step from conventional chemistry into chemistry-on-lab is reduced when using glass micromachining. Besides, glass shows excellent chemical and optical properties and allows a variety of design possibilities as demonstrated by the examples.

Furthermore, it should be kept in mind that in order to be able to fabricate (sub-) micron and nano-scale structures with high yield and quality, high demands are put on the purity and uniformity of the materials and surfaces and on the cleanness of the fabrication processes and fabrication environment, such that cleanroom environments and materials like silicon and glass become desirable, not only for prototype systems but also for commercial applications.

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