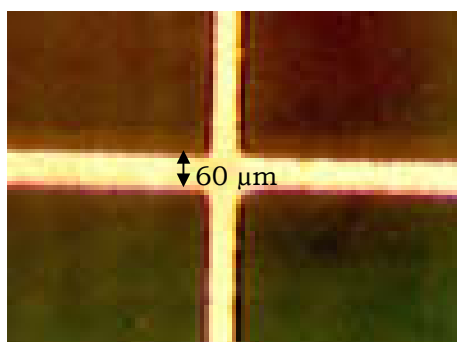


### 3.7.2. PLATE TRANSFERRED MASKS

To overcome both these problems (bad ink deposition and mask non-automatic handling) a technological process was developed to transfer high-resolution (3200 dpi) slide-film patterns onto commercial thin-film coated glass-plates that could be subsequently used as standard masks in the following photolithographic processes (see *Materials and Methods*, p.289). This basic procedure was later optimized by double exposure (with a low lateral displacement), both during slide-plate transfer and subsequent photolithographic processes, to reduce the occurrence of non-ink covered dots. This improved method produced high-resolution masks (more than suited for the purposes and motif-dimensions of this work, see Figure 54) that could be readily used at CNM-IMB clean room facilities with standard alignment equipment. Hence, this kind of masks was used in all the ulterior processes here described and, if not stated otherwise, references to masks imply by default plate-transferred masks across all the work here described.



**Figure 54** - RIE etched cross-injector in an electrophoresis chip after patterning with a plate-transferred mask.

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### 3.8. INSERTION/EXTRACTION DEVICES

As it has been previously stated, the need for access ports in DNA-chips is self-evident, as it is the general predicament that the interface with the macroscopic world poses in almost all  $\mu$ TAS. From early experiments, there was clear evidence that insertion and extraction procedures in DNA-chips were not a trivial issue. Although in some reports ([Burns1996] and, later, [Lin2000a]) the use of capillary effects had been suggested as the obvious means for the injection of reagents in PCR-chips, it was early assessed that the same principle did not hold true in the narrow-channels of electrophoresis chips, where superficial tension factors and the viscous

nature of some reagents (gels) held the capillary effect trick at bay. Moreover, even if reagent insertion can be carried out by capillary effects in PCR-chips, extraction of the maximum amount of sample (which is essential for ulterior electrophoresis analysis if quantitative detection is not integrated into the chip) requires either slight negative pressure in the outlet (vacuum suction, [Wilding1994]) or positive pressure at the inlet [Lin2000a], meaning that at least one of the access ports requires an airtight connection with an external pressure driven system. Due to the research nature, prototype-level, of this doctoral work, and the limited budget it operated upon, chip reuse (and therefore the availability of some method for astringent washing, see *Materials and Methods*, p.317) was seen as a major convenience and, thus, it accrued the necessity for reliable air/watertight coupled external pressure systems.

### **3.8.1. AIRTIGHT CONNECTIONS**

#### **Silicon technology approaches**

In view of these requirements, different approaches were studied to provide chips with airtight connections that would allow positive or negative pressures to be driven into the devices. A first, purely technological, approach was to engrave access holes on the silicon surface that could closely fit the typical pipette ends used in molecular biology. Unfortunately, the use of TMAH etching to open the access holes prevented this scheme, since wet anisotropic etching cannot produce round structures. Alternatively, the idea of creating a deposited circular ring surrounding the square-shaped hole was addressed, but it complicated an already complex process and required the special deposition of very thick resist layers, whilst it did not a priori guarantee tightness in the alignment of pipette tip and hole. Moreover, these technological approaches implied targeting chip design to a specific pipette tip (not a very flexible approach) and also required that pressure drivers (air or vacuum pumps) had to be somehow adapted to fit pipette tips.

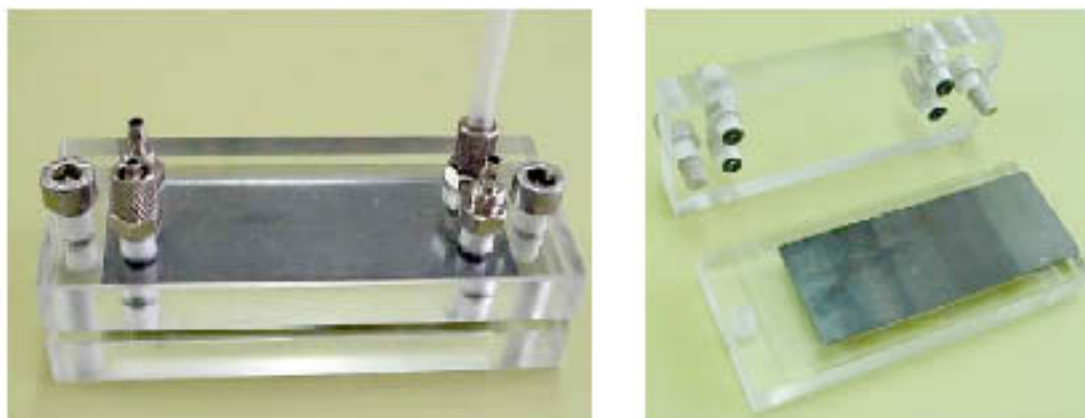
#### **Tube gluing and bonding**

A second approach, following the literature ([Wilding1994], [Poser1997], [Northup1997]), was to attach tubes or pipette tips directly onto the chip surface. This could be done both by using anodic bonding to bind a glass tube to the chip surface or by directly gluing a pipette tip to it. Anodic

bonding was discarded because it was predicted that the use of passivation layers, together with narrow tube diameters (as compared to tube lengths) would yield inefficient bonds that would be excessively fragile to the inevitable lateral stresses experimentation would bring forth. Similarly, pipette tips were glued onto the chip using standard contact glues, but the structures were also too weak to withstand the typical lateral stresses that could not be avoided when connecting the tips to a pumping source. Even if tensional forces could be reduced by coupling the tips (or tubes) to flexible pipes, it was assumed that the process would invariably generate fragile and cumbersome structures, with difficult prospects for mass production and associated problems due to the impossibility of correctly cleansing and/or sterilizing the insertion/extraction apparatus. Hence, it was decided to use an alternative and innovative approach, which consisted in the development of external insertion/extraction devices based on mechanization of methacrilate substrates.

### 3.8.2. METHACRILATE DEVICES

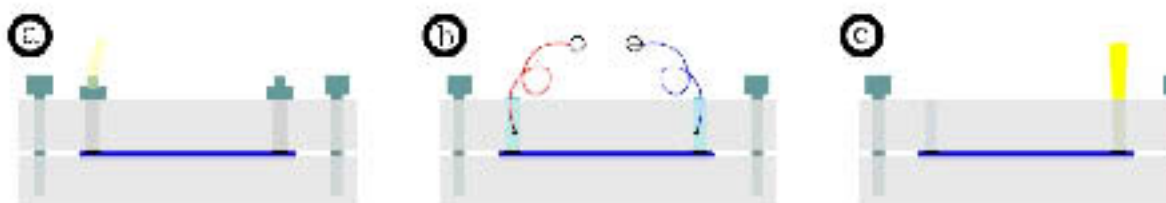
The methacrilate insertion/extraction devices basically consisted of a two-piece sandwich that wrapped the chip and could be effectively clamped using Allen screws (see Figure 55 and also *Materials and Methods*, p.299, for a full discussion on fabrication and design methodologies of methacrilate devices). Effective airtight junctions were accomplished by the inclusion of toric joints that were placed on top of the chip access holes and pressure clamped. To ease and make more reproducible the positioning of the chip with respect to the methacrilate holder, the chip outline was engraved on the surface of the inferior methacrilate slab by mechanical grinding.



**Figure 55** - Methacrilate washing device for serpentine-like PCR-chips.

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Following this methodology, various types of methacrilate devices (see Figure 56) were fabricated in order to provide different functional capabilities to the underlying chip. Injection and washing methacrilate devices were made by coupling  $\text{\O}6$  mm tube inlets at the superior methacrilate slab, allowing easy connection with  $\text{N}_2$ , air, de-ionized water and vacuum sources. With these devices, effective injection and removal of linear polyacrylamide gels for electrophoresis chips [Erill2000b] and efficient astringent washing procedures for PCR-chips (see p.193 and *Materials and Methods*, p.317) were demonstrated, with the advantage that the pieces could be washed, sterilized and rinsed separately from the chips. Additional methacrilate pieces with *open* holes were developed for electrode-placement in electrophoresis chips and reagent insertion and extraction on PCR-chips ([Erill2000c], see *Materials and Methods*, p.299). With these later devices, together with optimized designs (see p.133) for PCR-chips, very reliable insertions and extractions of PCR reagents and products were accomplished (with extraction yields well above the 75% barrier, see p.138), consistently improving those reported in the literature ([Lin2000a], [Lin2000b], [Zhan2000]).



**Figure 56** - Different methacrilate device designs for (a) washing with coupled 6 mm tube inlets, (b) open holes for electrode insertion or (c) pipette use.

### 3.9. CHIP DESIGN

As it has been stated earlier (see p.100-104), design methodologies, as capping strategies, have been generally disregarded, or poorly commented upon, in most PCR-chip papers. The obvious, initial conclusion after this fact is that either the basic scheme (a rectangular PCR chamber with two access ports, like that devised by *Wilding et al.* [Wilding1994]) is fully functional and efficient or, elsewhere, design (as capping) is not an important factor in PCR-chips. Indeed, a mix of both conclusions was the prevailing opinion at the beginning of this work, but it was early found out (and has later been confirmed by other researchers ([Lin2000a], [Zhan2000])) that this is clearly not so.

### 3.9.1. BASIC DESIGN CONSIDERATIONS

Prior to undertaking a discussion on design issues, the main outlines and restrictions of the designs approached in the present work must be laid out. Apart from the technological restrictions imposed on design by the already discussed technological options (as, for instance, the need to work with back-side, rectangular access holes) there were a set of design parameters (regarding, mainly, chip size) that were arbitrarily assigned within this work and require some justification. In principle (and in practice; see, for example, [Murakami2000]), PCR can be done in chips at the micro-scale, with reagent volumes in the pico-liter range, and the same holds true for electrophoresis chips if U-turns are included in capillary design [Jacobson1996]. Nevertheless, the effects of U-turns in electrophoresis fields and resolution are still not fully understood ([Seiler1993], [Jacobson1994]), and thus their use was discarded in initial electrophoresis experiments to prevent unexpected problems arising from U-turns, leading to the linear, centimeter-sized devices described elsewhere [Erill2000b]. Similarly, conducting PCR at the pico-liter volume was a novel technique that had not been previously contrasted using conventional instrumentation. Therefore, since the switch from eppendorf tubes to silicon chips was considered a fundamental enough change in the physical parameters of PCR, and bearing in mind the highly capricious nature of PCR (see p.69), it was decided to make chips that could muster reactions in the well known and eppendorf-tube checkable ranges of 25-50-100  $\mu$ l. This decision implied the use of deep etching techniques that, when the switch to double-side processing introduced 300  $\mu$ m wafers, obliged larger chamber areas and, consequently, large motif masks, all of them technological options that have already been detailed.



**Figure 57** - Gas-sensor chip

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### 3.9.2. DESIGN PROBLEMS AND EVOLUTION

The first PCR-chip experiments at CNM-IMB were done with chips borrowed from a gas-sensor project (TIC95-0978-C02-01) developed at CNM-IMB by *Jaume Esteve's* group of the Silicon Technology Department. These chips consisted in a 250  $\mu\text{m}$  deep micro-machined chamber of 10x10  $\text{mm}^2$ , providing 25  $\mu\text{l}$  reaction wells. The chips, created on a 300  $\mu\text{m}$  P-type silicon substrate, were sealed by anodic bonding with a pre-drilled 7740 glass wafer (*Pyrex*), presenting two access ports (see Figure 57). Even though the chips did not contain any passivation layer and thus exposed bare silicon to PCR reagents, they were deemed handy to provide early input on relevant PCR issues, such as insertion/extraction and capping procedures.

The results with these initial chips were concluding. Insertion and extraction procedures were carried out with a pipette tip and showed poor insertion and even poorer extraction yields (10-20% extraction). The main reason for this poor extraction was the close location of the access ports, which gave way to an easy formation of air-bubbles that impeded further extraction. Moreover, the positioning of the holes on the glass surface provoked easy breakage of the underlying silicon membrane when pressure was applied to securely fit the pipette tip.

#### **Redesign and methacrilate devices**

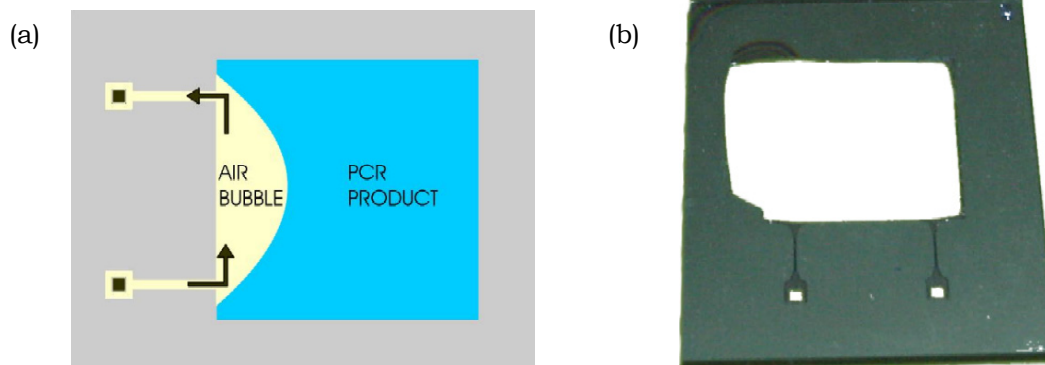
To overcome insertion and extraction problems, 50  $\mu\text{l}$  chips were designed with more separate holes, but, due to the larger chip surface, membrane breakage was a foremost problem, even during fabrication (see Figure 58). This new brand of chip designs was aborted and new prototypes were developed following the design ideas of *Shoffner et al.* [Shoffner1996], from which the basic insight was taken to append a couple of channels to the PCR reservoir leading to the access holes.

Using this scheme, physical pressure could be exerted onto the access holes without severe risk of breaking the reservoir membranes. Etching to a depth of 120  $\mu\text{m}$  (in 300  $\mu\text{m}$  wafers) to minimize membrane breakage problems, 25  $\mu\text{l}$  / 15x15  $\text{mm}^2$  reservoir chips were fabricated with two injection channels at one side of the reservoir leading to the access holes. Chips were then tested for insertion/extraction procedures using custom open-hole methacrilate tools (see p.128).



**Figure 58** - PCR-chamber membrane breakage during fabrication processes.

Although the results for reagent insertion were quite satisfactory (chips were easily loaded up to 80% capacity by simple pipette tip dispensing), extraction procedures remained still not up to scratch, yielding very poor extractions (between 15-25% of the inserted volume). The main problem came, again, from the formation of air bubbles at the reservoir wall connecting to the access ports, which prevented further extraction once it was set into place (see Figure 59), a fact recently noted by other authors [Zhan2000]. Chip inclination and overloading were assayed to prevent air bubble formation, but to no avail. To achieve higher extraction yields, chips were inserted into custom methacrilate devices connected to 6 mm tubing (see p.128), and negative (vacuum) and positive ( $N_2$  flow) pressures were exerted at one port, leaving the other port open. Nevertheless, even with this scheme, extraction yields did not rise beyond 40%, and there was the added problem of stuck reagents at tube walls. When, to force the remaining liquid out of the chip, both vacuum and positive pressure were simultaneously applied without leaving a ventilation port open, the 180  $\mu m$  reservoir membranes invariably broke under the stress (see also Figure 59).



**Figure 59** - Air bubble formation and airflow impeding extraction (a) and membrane breakage under vacuum plus positive pressure flow (b).

### ***Serpentine-like chips***

In view of the recurring problems for extraction of PCR reagents, it was decided to design a PCR-chip with serpentine-shape reservoirs. This scheme permits an easy insertion/extraction of fluids, since there is a unique path for air/liquid flow and thus, the formation of air bubbles, if they appear at all, does not pose severe problems on liquid extraction: reagents can be effectively drawn out of the chip with a slight positive pressure and the remnants, if any, can be further extracted by N<sub>2</sub> rinsing. This procedure had been previously tested and validated in capillary electrophoresis chips, where more viscous reagents were typically used [Erill2000b]. Regarding the negative effects serpentine-like structures might cast on PCR kinetics and efficiency, insight was taken from the already published work of *Manz's* group [Kopp1998] on serpentine-like PCR chambers, albeit the nature of constant-flow PCR did bring about different kinetic conditions.



**Figure 60** - Serpentine-like PCR-chip

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In their final version, serpentine-like PCR-chips (see Figure 60) were fabricated on 300  $\mu\text{m}$ , 380  $\text{\AA}$  SiO<sub>2</sub>-passivated wafers, with five interconnected 50x1 mm<sup>2</sup> channels etched down to 100  $\mu\text{m}$ , yielding ~25  $\mu\text{l}$  reservoirs (see p.137). Backside access holes were etched at both ends of the serpentine channel and the passivated chips were sealed with 1 mm-thick SD-2 glass wafers (*Hoya*), providing better bonding results than previous designs.