Microstructuring for Evolutionary Biotechnology and Molecular Information Processing

Spatial effects have been seen to play a decisive role in evolutionary theory since Darwin. Recent progress in our understanding of reaction-diffusion systems and in our technological capability to conduct molecular evolution experiments *in vitro* have prompted the design of experiments which unite these two advances in our lab. Most importantly, spatial effects appear essential in allowing the continuous optimisation of heterocatalytic function and in the optimisation and stabilisation of functionally coupled systems [1, 2]. The serial transfer technique commonly used in molecular evolution experiments, and further advanced in the SELEX procedure which has been used to evolve RNA catalysts, is, like the most common amplification method PCR, not suitable for an extension to the spatial dimension [3, 4]. The spatially resolved micro reactors we have developed are an extension of the flow reactor, an alternative method of maintaining constant amplification conditions with renewal of resources in the laboratory. A similar requirement arose in the study of Turing patterns in inorganic reaction-diffusion systems, where constant in- and outfluxes of chemicals are necessary to maintain the system far from equilibrium [5]. For biochemical experiments with evolving DNA however, much smaller volumes are required, contamination must be avoided and a higher level of integration associated with the combinatorial information is required. The reactions take place at low concentrations in the sub-micromolar range. This places special requirements on the miniaturisation and the associated sensitivity of detection that have led us to adopt the microstructuring technology in silicon and glass [6].

The aim of the microstructuring group is to develop techniques for the production of suitable microreactors for evolutionary biotechnology and molecular information processing. Special advantages of microreactors include sub-micro litre volumes, a high surface to volume ratio, integration of sampling, mixing, reaction chambers, detection and analysis of reaction products in a small area. Microstructured silicon and glasses offer the possibility of reaction vessels down to a resolution of 1 μm. The multiplication of the tiny structures on the same silicon wafer principally allows the parallelisation of the reactions up to about a million. The parallel transport, sampling and mixing of reaction solutions can be realised without contamination by using a closed microstructured channel system. Using anodic bonding, closed reaction vessels can be produced for millions of reactions and a total volume of < 10 μl. A further advantage of closed reaction vessels is the avoidance of evaporation of the small volumes. The material transport can be controlled with the help of controlled pressure, micro valves and electrical fields. Modification of the surfaces of the reaction chambers or channels and the controlled distribution of beads allow the specific immobilisation of enzymes at the structured surfaces. An integrated reactor concept will include sensors and actuators in thin layer technology which serve to control the temperature and to transport the solution and/or its contents. Time control of molecular sorting, down to the single molecule level is possible: one strategy requiring the production of micro electrodes in the microstructures [7, 8]. Micro separation layers can be produced by filling polymers into the structures. With the help of micro pumps and valves, material flows are to be produced and controlled [9].

The material conventionally applied in the field of microstructuring is silicon following its success in integrated circuit manufacturing. Photolithographic techniques make it possible to produce many copies of similar structures, size and quality down to the submicron level. The layout of a structure can be designed on a PC or workstation and transformed into a photomask,. An image is transferred to a photosensitive compound (photoresist) spun to a film over the silicon wafer. When the illuminated parts of the resist have been removed (the development process is similar to film development in photography) it is possible to start etching the uncovered parts of the wafer. This well established technology is highly flexible, allowing changes to the design as well as allowing many copies to be produced in one design. A further advantage of using silicon and glass is the sealing without adhesives of silicon to silicon by silicon fusion bonding [10] or sealing silicon and glass by anodic bonding [11] in order to create a broad range of three dimensional reaction vessels.

From Capillary Microreactors to Simple Flow Reactors

Experiments in closed capillaries lead to travelling concentration waves with evolving RNA, and parallel observation allows statistically significant conclusions to be drawn [12, 13]. Evolution and selection experiments develop a spatial aspect: selection takes place in travelling wave fronts (places of rapid growth) and the speed gives information about the amplification rates. For these travelling wave experiments, a microstructured parallel capillary reactor provided a first target application requiring only the three basic microstructuring steps of etching, bonding and connections. In these microstructured capillaries in silicon and glass basic investigations about surface effects and special surface coatings, the fluorescence intensity of the submicromolar concentrations of DNA and the fluid handling in the closed microstructures could be carried out to establish a biochemically usable technology.

For silicon microfabrication, 4 inch <100> p-type silicon wafers were used. After a photolithographical process, the resist structures produced are transformed into 1 μm thick silicon dioxide layers, which are commonly used as a mask for deep silicon etching. We employ both isotropic and anisotropic etching solutions for deep silicon etching to produce channels and reaction chambers in silicon [14]. For the structuring of pyrex glass a technology was developed which allows the etching of channels and meander structures up to a depth of 20 μm into the glass by using chrome masks on the glass surface and applying an isotropic etching solution. By this means, interconnection and distribution channels from one silicon channel to the other can be achieved in anodically bonded pyrex glass. Following the structuring of silicon and glass wafers, they can be sealed to one another by anodic bonding. Using anodic bonding a tight connection can be achieved without adhesives and closed reaction chambers can be manufactured.

In order to pump fluids into a single channel or meander, a technology was developed which allows us to connect a plastic or glass capillary directly to a silicon channel. The microstructures can then be filled with biochemical solutions and drained. Holes were produced through the cover pyrex glass wafer via ultrasonic drilling. A glass capillary with an inner diameter of either 50 μm or 400 μm was directly connected to the silicon structure. The capillaries are held by a PMMA bar which is directly glued to the glass wafer. Figure 1 shows a silicon wafer with connected plastic capillaries.

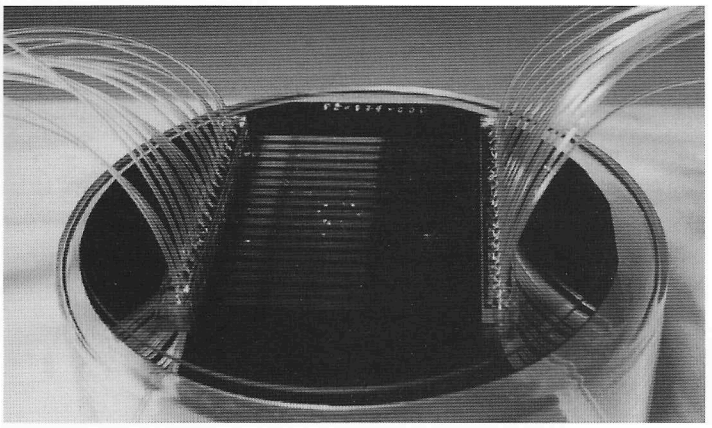


Figure 1: Photo of a 4" silicon wafer bonded with pyrex and containing 17 capillary reactors with connected plastic capillaries (see text).

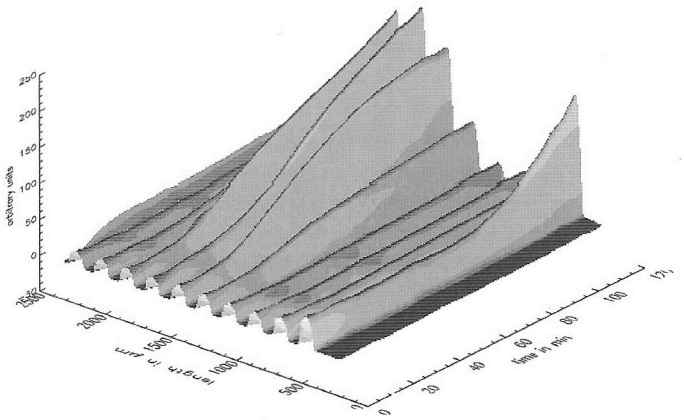


Figure 2: 3SR amplification in microstructured travelling wave reactor. The fluorescence intensity of To-Pro-1 labelled DNA was taken with a cooled CCD-camera at intervals of 1 minute. The images were integrated for each turn of the meander before being plotted, so the length axis shows successive lengths of the capillary.

A further method of making microfluid contacts to microstructures is the connection to microchannels from the back of the silicon wafer. In addition to the structures for the flow reactors, holes are etched through the silicon to connect the channels from the back side of the wafer. The plastic or glass capillaries are fixed to the back side of silicon wafer, as described above. This technique requires more room (ca. 800 μm) because of anisotropic etching angles, but is less labour intensive than ultrasound drilling for many connections. The technology developed to connect microchannels to external capillaries is easy to realise, very flexible to add modifications into the design and allows to connect a large number of reaction chambers.

We developed layouts for a capillary reactor on the basis of anisotropic etching of silicon and subsequent bonding with pyrex glass wafers (chosen to minimise thermal expansion effects). Individual meander structures were etched into silicon with a length of 0.5 meters. For this test phase, 17 meander structures were arranged on one silicon wafer.

The 3SR *in vitro* amplification reaction [15, 16] was tested in this capillary reactor. The meander was filled with unreacted 3SR reaction mix and the microstructure warmed to 37°C in order to start the reaction in the channels. It was demonstrated that inhomogeneous growth along the

length of the capillaries could be successfully detected. Figure 2 is an example of the intensity distribution at different points of the meander. The experiment shows that the observed fluorescence intensity is sufficient for travelling wave experiments in microstructures. A higher level of integration can be reached using silicon microchannels instead of plastic capillaries.

A further development of the simple capillary reactor is represented by the pico-drop-reactor. Millions of separated reaction volumes on the sub-nano-litre level can be manufactured serially via microstructured T-junctions and capillary meanders. The separation is carried out in thin channels by applying an inert gas such as argon. Hydrophobic channel walls stabilise the moving separated droplets. For the production of such a pico-drop-reactor, meanders were etched in silicon and T-junctions were etched in glass perpendicular to the meander. Here, the isotropic etching of glass developed in our group was applied.

Flow Reactors with Mixing, Immobilisation of Beads and Velocity Control

Usually one photolithographical step allows only the structuring of one channel depth. For the realisation of barriers to hold back beads, for the combination of chambers and channels or for reactors with different velocity zones it was necessary to structure silicon with different structural depths. For a flow reactor with a barrier to hold back beads a grid of capillaries with a width of 20 μm and a depth of 10 μm should be connected to a reaction chamber with a depth of 200 μm. These extreme differences in the structural depth cannot be realised with just one etching process. It is necessary to produce reaction chambers in a first step and the capillaries in a second. After the first etching process, a further photolithography process is necessary to structure the mask material for the second etching process. Traditional methods do not allow an even application of photoresist in the presence of deep structures, as during the spinning the resist breaks at the structure edges and accumulates there, preventing an even exposure and development of the resist. The procedure which we developed makes multiple structuring possible, with different etching depths. The process of etching the mask material (silicon dioxide) was modified in such a way that further photolithography after the first anisotropic etching is no longer necessary.

The etching process for multilevel structuring was optimised so far that the first structure can be completely etched through the whole wafer. Later, these structures which are completely etched through the whole wafer can be used for the transport of biochemical material to the back of the wafer. This allows a connection of the channels to external plastic capillaries. Two further structure levels are produced as described above. For this technology it is necessary to apply three processes of photolithography in order to reach three different stages in the silicon dioxide.

The following reactor types were produced by using the technology described above.

A) Parallel Microflow Reactors

Traditionally biochemical reactions are studied in batch despite the fact that many important reactions are adversely affected by the fast consumption of the reactants and the inhibition of the products. This also applies to *in vitro* amplification processes such as the 3SR-reaction. As a move towards a chemically open spatial microreactor and actively controlled flow processors an intermediate step via stirred microreactors was chosen.

With magnetic beads, microvolumes of several μl and smaller can be stirred in order to enhance the diffusion exchange which is very fast on this scale. A grid of capillaries (20 μm channels) restrains the beads (e.g. 30 μm) under flow. The time scale is represented spatially via a meander which is linked behind the microreactor. Enzymes immobilised on beads can be pumped into the reaction chamber and there they are held back through the capillary array. Such a module of reaction chambers, sieves and analytical meanders has been realised with a width of less than 2 mm. Higher levels of integration are possible. Figure 3 shows the SEM-picture of such an etched flow reactor.

After the wet silicon etching, the silicon structures were sealed with a pyrex wafer to create a closed reaction chamber. Narrow channels behind the reaction chamber act as a barrier for enzyme loaded beads. In order to pump fluids into the reactor, the whole microstructured reactor has to be connected to an external pump using plastic capillaries.

With the masks employed, the depth of the reaction chamber can be etched between 50 μm and 200 μm deep, depending on the application. In this mixing reactor a co-operatively coupled system was investigated [17].

B) Planar Flow Reactor

In molecular biology open, spatially extended reactors have been almost completely neglected in the past. For the evolutionary biotechnology such reactors offer the possibility of optimising the heterocatalytic properties of

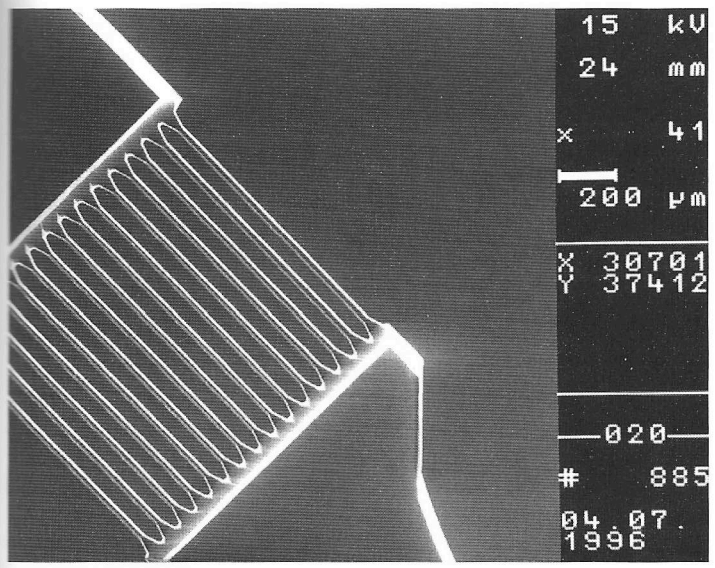


Figure 3: SEM-picture of a part of the microflow reactor (see text), this grid of capillaries restrains the beads from leaving the reaction chamber (top left)

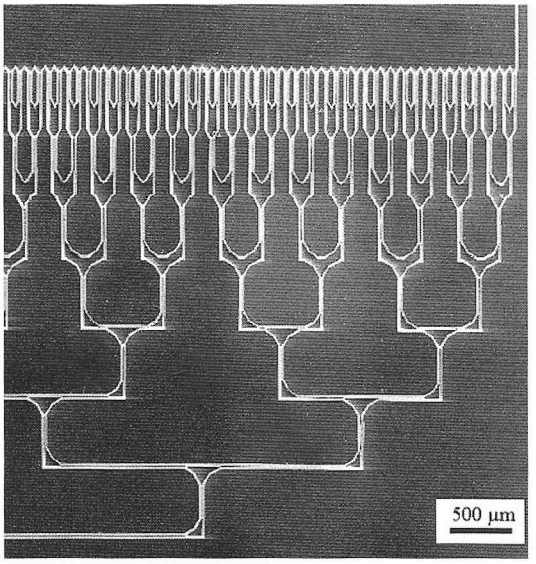


Figure 4: Photograph of the bifurcation filling structure (anisotropically etched silicon version). The filling channels are 40 µm wide and the number of level in the hierarchy is adapted to the width of the reaction chamber

molecules. Due to the otherwise high consumption of enzymes and primers and to contamination problems, this development can only be made acceptable using microreactors. A silicon-glass-bonding structure can be used as the simplest version of such a microstructured reactor. A homogeneous flow profile over a width of 1-7 cm with flow rates suitable for amplification (ca. 1 µm/s) can be realised with bifurcated capillary structures. The width and the depth of the reactor chamber can be varied. Immobilised enzymes can be placed on the floor or on the cover glass of the reaction chamber, or immobilised on beads which can be held back by a capillary barrier as above.

Together with Dr. Foerster and her student Arne Bochmann we have developed a design for this type of flow reactor. A homogenous flow is achieved by means of a bifurcation structure at the entrance and exit of the reaction chamber, see figure 4. The flow characteristics in the chamber could be detected using fluorescent beads.

The cascaded bifurcation structure alone allowed Bochmann to perform several additional reaction studies. One is the investigation of the diffusive behaviour of the reactants. A simple modification of the bifurcation cascade, providing it with two supply channels, allows one to fill each half of the reaction chamber with different reaction mixtures. Owing to the highly laminar flow, a sharp boundary between the two different reaction mixtures can be created. After stopping the flow, diffusion takes place as observed by fluorescence spectroscopy. Using different reaction mixtures, the diffusion behaviour of DNA, RNA and enzymes complexes were observed. Travelling reaction wave fronts could be created from sharp initial profiles.

C) One Dimensional Open Microreactor

Using the bifurcation structure as described above, a one dimensional open microreactor with a length of 6 cm can be realised. 1024 channels enter into the reaction chamber, which can be mixed locally by diffusion, on the timescale of 100 s which is the residence time, width and breadth being ca. 100 µm. To prevent back contamination under a range of flow rates the flow velocity in the inlet structures is about a factor of 100 larger than in the reaction chamber. This can be achieved by multilevel etching of 20 µm wide and 10 µm shallow inlet structures. This reactor allows continuous spatially resolved evolution experiments to be investigated. Figure 5 shows a photograph of one end of the reactor.

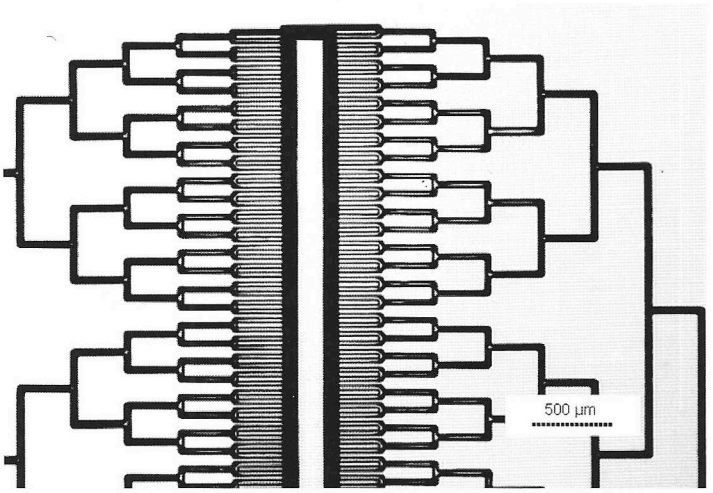


Figure 5: Photograph of one end of the one dimensional open reactor. The reaction chamber forms a long (vertical) one dimensional open media for spatial biochemistry. Flow is from left to right.

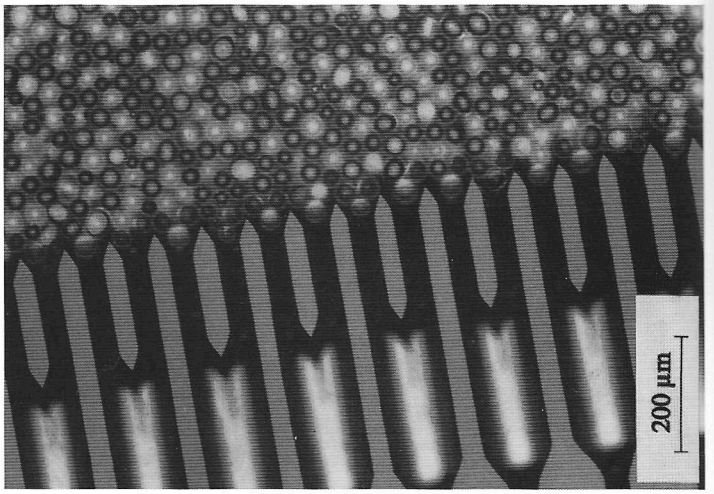


Figure 6: Holding back beads for enzyme immobilisation in a flow. The reaction chamber (top) is filled with polystyrene beads. In this case, the channels are 40 µm wide and have a V-shape: they are able to hold back beads larger than 40 µm, which are restrained without blocking the outflow (below).

D) Restraint of Beads in Spatial Microreactors

A continuous renewal of enzymes for long-term experiments is very expensive. In order to reduce the consumption of enzymes significantly, they have to be immobilised in the reaction layer. Two methods are possible: to immobilise the enzymes directly on the surface or to immobilise the enzymes on beads first before loading them to the reaction layer. If enzymes are immobilised on beads, structures have to be developed which hold back the beads without interfering with the flow. We produced structures which on the one hand perform this function but on the other hand allow the chambers to be filled with beads.

The structure consists of one chamber as well as entry and an exit structure. The entry and exit have been shaped as a distribution structure. The entrances have been made wide enough to enable the beads to enter into the chamber without obstruction. The width of the channels on the side of the exit are smaller than the beads, in order to hold them back. Figure 6 shows a capillary structure with beads being held back.

Microreactors for Single Molecule Detection

The ultimate level of integration of molecular information processing is at the single molecule level. Single molecule detection in microstructures places specific requirements on the microreactors. The objectives we are using for detection have been corrected to a thickness of cover glass of 170 µm. So it is important that the glass used to close the structures via anodic bonding has the same thickness. The technologies applied for the coupling of filling capillaries to the wafers also have to be adapted to this thickness.

The main goal is to minimise the background noise which is caused by reflecting silicon. We developed a technology which allows us to produce the bottom side of a channel in silicon dioxide. Channel and meander structures are etched in silicon and after the anisotropic etching they are covered with a 3 µm layer of thermal silicon dioxide. The wafers were bonded with pyrex glass and the silicon of the silicon glass bond is etched again anisotropically from the reverse side until the silicon dioxide of the channel floor has been reached.

The channel and meander structures produced with this technology are transparent on two sides. The transparency at the top is achieved by the pyrex glass, at the bottom by the 3 μm layer of silicon dioxide. Figure 7 represents a channel structure which was produced according to this technology.

In these microchannels single DNA molecules with a length of 8000 base-pairs were detected. A zero-dimensional confocal set-up was used. The DNA molecules were labelled with an intercalating dye [18].

The detection of single molecules in a silicon channel provides a basis for further development towards spatially resolved single molecule detection in microstructures. In co-operation with the IPHT Jena a molecule sorter has been planned and first designs drawn up.

Two Dimensional Vertical Flow Reactor

Experiments in two dimensional gels have demonstrated circular reaction-diffusion fronts which can be used to monitor evolution, but such systems amplify to fill the reactor and the process stops when all resources are consumed. Such closed reactors are only suitable for the investigation of transients. The achievement of a homogeneous flow of chemicals perpendicular to a two dimensional thin layer requires special hydrodynamic calculation and measurement. In contrast with the inorganic case, the reactor must be constructed in such a way that lateral contamination by product DNA is avoided, since this would introduce non-local effects into the evolution. Flow rates must be adjusted to the slow rates of biochemical polymerisation and diffusion with concentration waves in the micrometer/sec range.

In collaboration with Dr. Foerster an open, two-dimensional flow reactor for the spectroscopic detection and sampling of the amplification products was developed. The flow reactor allows the continual supply of reactants as well as the simultaneous removal of the reaction products. A perpendicular flow through the reactor permits the homogeneous supply of the reactants (dNTPs, rNTPs, primer), the distribution of the reaction templates for the initiation of the reaction and the removal of the reaction products (RNAs, single-stranded and double-stranded DNA). Such a reactor type could not be realised in a single wafer. To reach the homogeneous flow through the active layer two wafers which act as a pressure barrier and two wafers for filling and outflow are necessary. At the moment the reactor consists of 7 wafers. Silicon direct bonding and anodic bonding were employed to bond all wafers together. Complex three dimensional multi-layer sandwich structures can be realised.

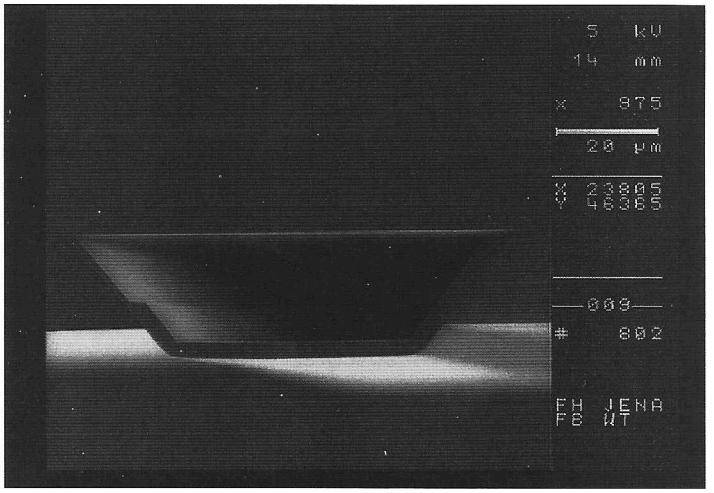


Figure 7: SEM-image of a bonded channel structure with silicon dioxide window (cross section) for single molecule detection. The SiO2 window protrudes below the plane of the channels.

The reactor has a modular sandwich structure. The centrepiece of the microfabricated reactor is a two dimensional active layer, carrying the enzymes necessary for the reaction. The lateral spatial dimensions, motivated by the dimension of the expected spatial patterns ($\leq$ 1 mm), are on the scale of centimetres. The vertical dimension is at present on the scale of 200 μm, making this structure particularly suitable for the detection of two dimensional reaction patterns.

A bifurcated filling structure and a microstructured sieve above and below the reaction layer are used to generate the homogeneous perpendicular flow. The filling layer consists of a chamber and two opposing bifurcation cascades. One of these layers provides homogeneous supply of the reactor with the reactants and the distribution of the templates for the initiation of the reaction, whereas the layer on the top guarantees the removal of the reaction products.

The microstructured sieves are made by etching narrow channels in <110>- silicon wafer. When immobilising the enzymes on the surface of

the reaction chamber, a standard 200 μm thick glass wafer can be used as reaction chamber. In the other case, when using beads for the immobilisation, the active layer needs channels for the input of beads into this layer [19]. The multi-layer structure is sealed by bonded glass underneath and on top. The scheme of the reactor is shown in figure 8.

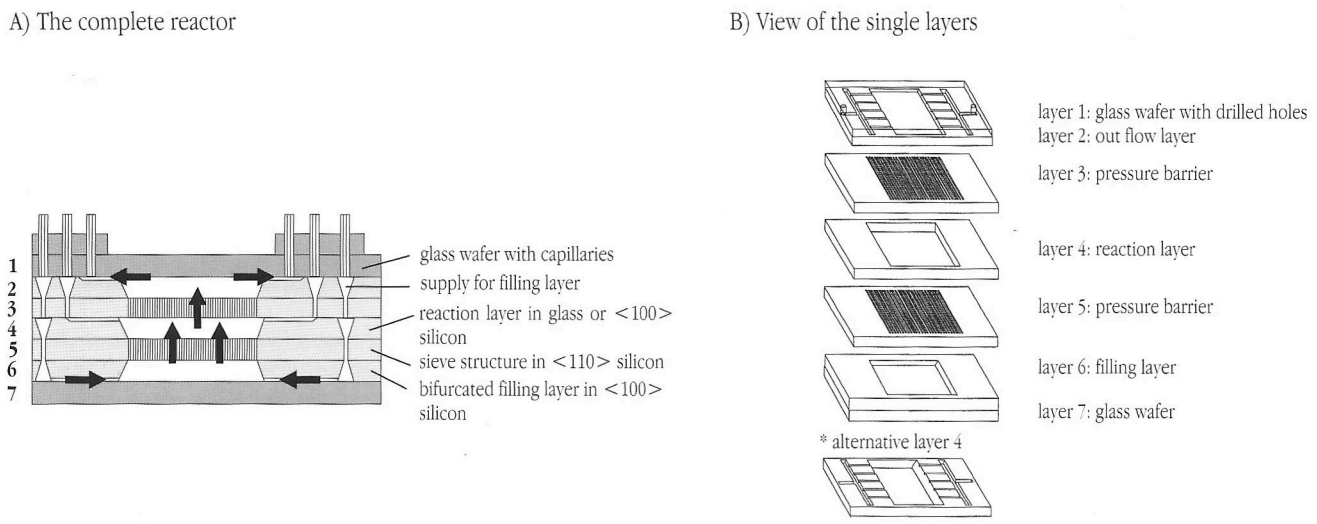
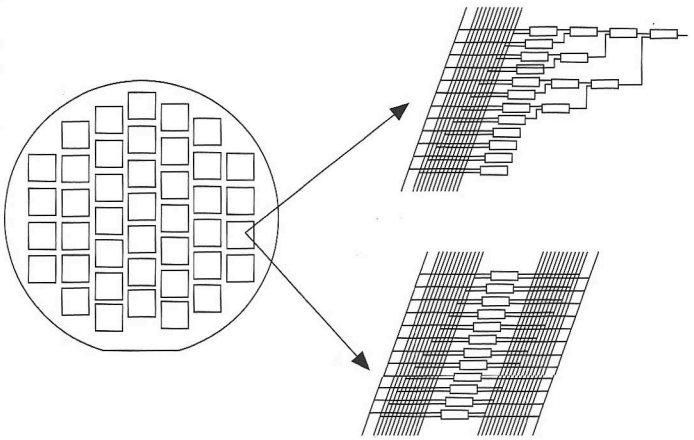


Figure 8: Reactor design of the two dimensional vertical flow reactor, consisting of 7 layers
A) schematic drawing of the complete reactor, B) each layer in detail (see text). In the central reaction layer it is possible to observe fluorescence through the transparent pressure barrier.

Active Controlled Microreactors

The next step towards a fully integrated evolutionary biotechnology involves an intelligent feedback from monitoring equipment to actively control the course of reactions in the microreactors. In microflow reactors this starts with simple overall flow control, which has been achieved with computer controlled syringe pumps, homogeneous temperature control via resistive electrical heating elements and voltage of external electrophoresis electrodes. A second stage of parallel local control, allowing combinatorial processing, requires the integration of local active elements such as microvalves, micromixers, microheaters and microelectrodes. In conjunction with bead microfluidics a rather powerful highly integrated processing facility can be achieved. At the one extreme is the target of a million active microvalves to achieve a switchable microflow reactor capable of computer mediated optically detected intelligent flow control of thousands of parallel reactions. Such numbers are motivated by the need to screen the combinatorial complexity of possible structures and reactions of evolving biomolecules. At the other limit is the desire to proceed to the single molecule level of control. Single molecule detection and sorting provide the ability to associate function with structure in the heterogeneous evolving populations required for optimisation. Our approach towards this limit is to develop first a single channel molecular sorting facility before proceeding to the massively parallel integration of the former goal. Although our single molecule sorting facility, developed together with the IPHT (Dr. Schultz), is quite far advanced, integrating novel two dimensional detection with novel

Figure 9: Basic designs for integrated combinatorial microflow reactors. Left: package density of structures on a 4" silicon wafer. Right: Combinatorial mixing module with cross-over channels in <110>silicon. Below: single level, Top: multilevel



fluid control mechanisms, we shall leave this subject for a separate article. Instead, we wish to outline the benefits of a parallel active flow reactor concept for evolutionary biotechnology.

The basic design for integrated combinatorial microflow reactors is shown in Figure 9. The reactor elements resemble the components of electronic chips: the microchannels which can intersect or cross-over correspond to connecting wires, the microvalves to flip-flops and the microreaction chambers to gates. An equivalent to a Field-Programmable-Gate-Array (FPGA) structure of configurable hardware can be achieved using microvalves to open and close microchannels and micromixers to turn mixing on and off. Control of these individual elements is achievable via piezoelectric crystals or exploiting magnetic particles. Steady flow pressures (cf supply voltage) can be achieved using off-chip sources such as pumps or using narrow outlet chambers and/or microheaters. A high level of integration including channel crossover can be achieved in <110>Si and two sided etching as shown. Parallel deep narrow channels, horizontal on one side and near vertical on the other provide the cross-over channels, with individual through-etching sites implementing a contact. The latter may be opened and closed by microvalves. Chip modules with the order of 100 external capillary connections and thousands of channel crossovers and reaction chambers allow detailed control of combinatorial link-up of amplification and enzymatic or chemical manipulation steps for parallel evolutionary processing. The ultrasensitive fluorescence monitoring with cooled back-illuminated CCD chips and image processing allows a real-time intelligent control of these many active elements. First integrated modules will be available in 1997. This development provides the microreactor development for an evolvable hardware and can be linked up with configurable electronic hardware components to provide new hybrid evolution possibilities.

Membranes in Silicon

In the remainder of this section we describe the basic silicon membrane structuring technology developed by R. Bräutigam in our group, which forms a basis for integrated active microvalves and other components.

For the realisation of active components, membranes with a thickness smaller than 10 μm have to be produced. Therefore it is necessary to thin down the used bulk material which is in silicon in this case. Employing anodic oxidation of silicon the etching process can be stopped at a pn-junction. A n-epitaxy layer (thickness 5μm) is deposited on a p-doped silicon bulk material. The etching process started at the p-material. When a voltage is applied while etching, the etching process stops at the pn- junction and the n-epitaxy layer is preserved. The advantage of a silicon membrane compared with membranes of SiO_2 or Si_3N_4 is that they have the same expansion coefficient as the bulk material and that leads to a considerable increase in the stability of the membranes.

A device was developed which allows the controlled fabrication of silicon membranes with a reproducible thickness. The membranes will be applied as movable parts for micro valves or micro pumps. A 500 μm x 500 μm membrane can be deflected up to 8 μm. This flexibility can be used for opening or closing a microvalve. Integrated microvalves allow the active control of the flow in a micro fluidic system. In another application the thin silicon membranes act as sieves. In this case an additional etching step produces holes in the micrometer range into the thin layer. Such mem-

branes are stable enough to allow fluids to be pumped through. The structured membranes can also be used as inlet parts for filling elements, using surface tension effects [20].

The clean room facility of the IMB, as documented in this article, now provides a unique platform for biochemical microstructuring applications in molecular biotechnology.

Acknowledgment

This work was supported by a grant from the German ministry of education, science, research and technology (BMBF 0310799-0310805).

Kristina Schmidt, John S. McCaskill
Molecular Information Processing

References

[1] M. C. Boerlijst and P. Hogeweg. Physica D 48 (1991) 17-28

[2] J. S McCaskill. In: Theory & Control of Dynamical Systems (Andersson, S.I., Andersson, A.E. & Ottoson, U., eds.), World Scientific, Singapore (1991) 1 - 28,

[3] K.P Williams and D.P. Bartel. In: Catalytic RNA (Eckstein, F., & Lilley, D.M.J., eds.), Springer, Berlin (1996) 367-381

[4] M. A. Innis, D.H. Gelefand, J. J. Sninsky and T. J. White. A Guide to Methods and Applications, Academic Press, San Diego (1990) 482

[5] V. De Kepper, E. Castels, E .Dulos and J. Boissonade. Physica D 49 (1991) 161-169

[6] K. Schmidt, P. Foerster,A. Bochmann and J. S. McCaskill. Proceedings of IBC´ Conf. of microfabrication and microfluidic technologies, San Francisco (1996)

[7] A.T. Wolley and R.A. Mathis. Proc. Natl. Acad. Sci. USA, Vol. 91 (1994) 11348-11352.

[8] M. A. Northrup, B. Beemann, D. Hardley, P. Landre and S. Lehew. Proceedings of IBC´ Conf. of microfabrication and microfluidic technologies, San Francisco (1996)

[9] P. Gravesen, J. Branebjerg and O.S. Jensen. Journal of Micromech. Microeng. 3 (1993) 168-182.

[10] Q.-Y. Tong, G. Cha, R. Gafiteanu and U. Gösele. Journal of microelectromechanical systems 3 (1994) 29-34.

[11] M. Harz. Journal of micromechanics and microengineering 2 (1992) 161-163.

[12] G. J. Bauer, J. S. McCaskill, and H. Otten. Proc. Natl. Acad. Sci. USA 86 (1989) 7937-7941.

[13] J. S. McCaskill and G. J. Bauer. Proc. Natl. Acad. Sci. USA 90 (1993) 4191-4195.

[14] Y.Bäcklund and L. Rosengren. Journal of micromechanics and microengineering 2 (1992) 75-79.

[15] J.C. Guatelli, K.M. Whitfield, D.Y. Kwoh, K.J. Barringer, D.D. Richman and T. R. Gingeras. Proc. Natl. Acad. Sci. USA 87 (1990) 1874-1878.

[16] B. Wlotzka, J.S. McCaskill. Chemistry and Biology 4 (1997) 25-33.

[17] R. Ehricht, T. Ellinger and J. S. McCaskill. Europ. Journal of Biochemistry 243 (1997) 358-364.

[18] B. Wagner, H. Mathis, K. Schmidt, G. Kalusche and J.S. McCaskill. Nucleosides and Nucleotides 16 (1997) in press

[19] K. Schmidt, P. Foerster, A. Bochmann and J.S. McCaskill. Proceedings of 1st International Conference on Microreaction Technology, Frankfurt (1997)

[20] S. Howitz and M.T. Pham. DE 445005 (1994)