# LAB-ON-A-CHIP AND MICROREACTORS: THE MAGIC WORLD OF MICROFABRICATED DEVICES

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The interest in microfabricated devices for performing chemical and biological assays appears to be in exponential phase of growth. We can compare the advancements in microfabricated devices with the remarkable evolution of the Microelectronics and Information Technology (IT) sector[1].

Considering the number of human gene products and the literally millions of compounds, such as oligopeptide sequence, which can be tested for gene-specific activity, the total number of experiments for potentially useful drug is enormous. For such large-scale, experimental endeavours to be tractable, a technological shift from the present-day laboratory may be necessary. Micro-fabricated fluidics may represent the enabling technology capable of providing the necessary quantum leap in experimental power.

Consider the complex chemistry undertaken by cells and bacteria, which mediate chemical processes at micro rather than the macro scale. In such evolutionary successful life forms, chemistries are efficiently controlled without need for many of complex practical methods found in human laboratories. Why then do we humans not carry out chemistry at that scale used in nature and so gain advantages such as control, speed and selectivity of chemical processing enjoyed by humble bacteria?

**Why use microfabricated devices ?**

There is one more similarity in the development of microfluidics and microelectronics i.e. their fabrication i.e. photolithographic patterning tools that originated in the microelectronics industries are often used to produce microfluidic devices. The "carriers" in microfluidic system are liquid reagents flowing through a fluidic circuit, rather than electrons flowing through semiconductor circuit, the miniature scales of both systems offer analogous advantages e.g. in microfluidic circuits molecules travel shorter distance just as electrons do in microcircuits, thereby speeding up processing. This is advantageous in chemical separation devices and microreactors, where reagents have to be mixed. The small dimensions also reduce the amount of reagents necessary to perform a chemical process for a single experiment. Volumes are often in the nanolitre to picolitre range rather than the microliter range or even larger in conventional experiments. The photolithographic fabrication of microfabricated devices potentially can provide number of advantages. Devices containing highly replicated channel structures can be fabricated with small incremental cost per channel. Such economy of science will allow manufacture of inexpensive disposable devices for many applications such as research and clinical diagnostics. In modern scientific language the microfabricated devices used for such research and clinical diagnostics or clinical analysis are termed as "Lab-on-a-chip".

Microfabrication techniques advanced a lot and there has been a spectacular advance in the electronics industry, and they are now creating new opportunities for reaction engineering. Microsystems have feature sizes in the micron to hundreds of micron range, the reaction components are usually integrated with sensors and actuators. The reduction in size and integration of multiple functions create structures with capabilities more than those of conventional macroscopic systems. They also add new functionality while preferentially making low cost, mass production possible. Existing examples are nucleic acid synthesis and detection, and microchemical analysis, combinatorial synthesis of drugs and microchemical reactors for on-line demand synthesis.

These developments are due to advances in MicroElectroMechanicalSystems (MEMS). The research investment made in MEMS has enabled the fabrication of microchemical reaction synthesis. Miniaturisation of chemical analytical devices in 'Micro-Total- Analysis-Systems' (μTAS) represents a natural extension of MEMS technology to chemistry and biology with obivious applications in combinatorial chemistry, high throughput screening, and portable analytical measurement devices.

## Methods Of Manufacturing Microfabricated Devices

Ever since the microelectronics industry began manufacturing silicon chips, the techniques for manufacturing microreactors have potentially been available. Currently , microreactors are made in number of ways, and many different materials are used. Devices produced from metals, glass and silicon are common, mainly because of their chemical inertness and temperature stability. For example the chemical stability of silicon based devices means that they can withstand very harsh cleaning regimes, such as presence of Oxygen at 1000 °C .

There are a number of methods for micromachining silicon, including photolithography, and isotropic and anisotropic etching. An important fabrication technique for metal microreactors is microlaminations. Thin laminates of metal are stacked to give the channels and partitions. There are number of simple methods to cut channels into metal laminates, including soldering , brazing, high temperature diffusion bonding, and adhesive bonding.

### Construction Techniques[6]

**Etching :** Until the more recent trend towards using polymeric substrates, chemical chips used glass, quartz or silicon, sometimes with patterned metallic or polymeric structures. Thin film techniques are usually used to put the chip with a patterned mask that resists a subsequent subtractive process such as etching. A variety of etching techniques exists that gives rise to characteristics geometrical profiles. Wet etching of glass, typically using HF acid ammonium fluoride usually produces a semicircular profile, while in silicon the profile depends on crystallography. This profile, which results from material beneath the mask being eroded (see figure) limits the definition obtainable by this technique.

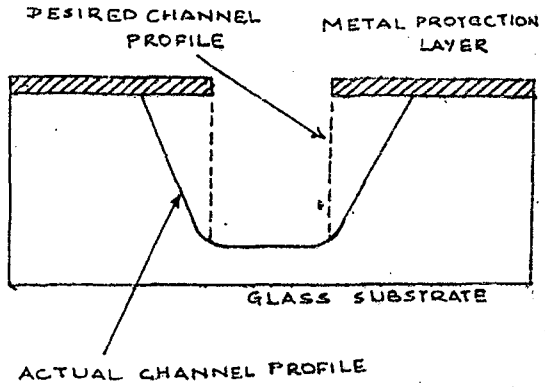



**Figure 1: Wet etching : As the channel is etched deeper, the width is increased by the action of etching solution on the exposed walls of the channel.**

Anisotropic etching processes, which use directional beams such as in deep reactive ion etching do not suffer from this problem and are useful where high aspect ratio structures are needed. Glasses with solubility in acid can be altered by differential exposure to light(Photo structurable glasses have been explored for microfluidic applications. They are too costly and leave a surface roughness which are not acceptable in many chemical applications.

**Laser microforming:** Direct laser ablation (see figure) [6]is an emerging micromachining process that has wide applicability for making chemical chips. It uses pulses of ultraviolet laser light , to break molecular bonds in the exposed material & form channels. Material can be removed at controlled depth intervals of less than 100nm.

Usually excimer lasers-based on light emitted from the decay from an excited dimer-working at 248nm and 193nm are used, although recent developments in F2 (fluoride) lasers working at 159 nm should allow more rapid micromachining of inert materials such as fluoropolymers, which are often preferred for chemistries involving solvents.

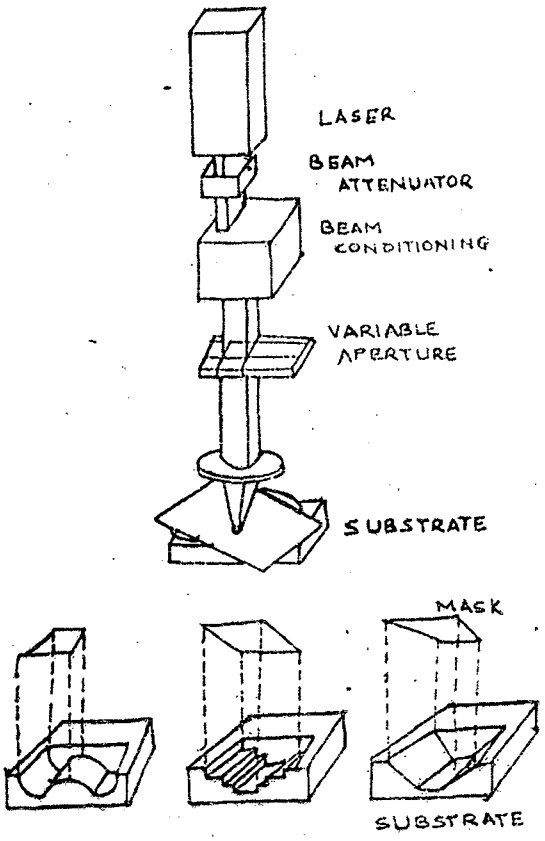



**Figure 2: Laser microforming**

A major advantage of this technique is that the laser does not heat the materials ,avoids heat damage,which makes it very useful for removing plastics.In addition ,excimer laser micromachining can rapidly generate prototypes, so designs can often be turned from drawing into prototype within few days. This technique is applicable to any material that will allow molecular bonds to be broken with exposure to high peak energy UV radiation. Also this technique Extends to mass production using reel-to-reel processing & is currently used for ink-jet printer heads & microtiter plates, for example.

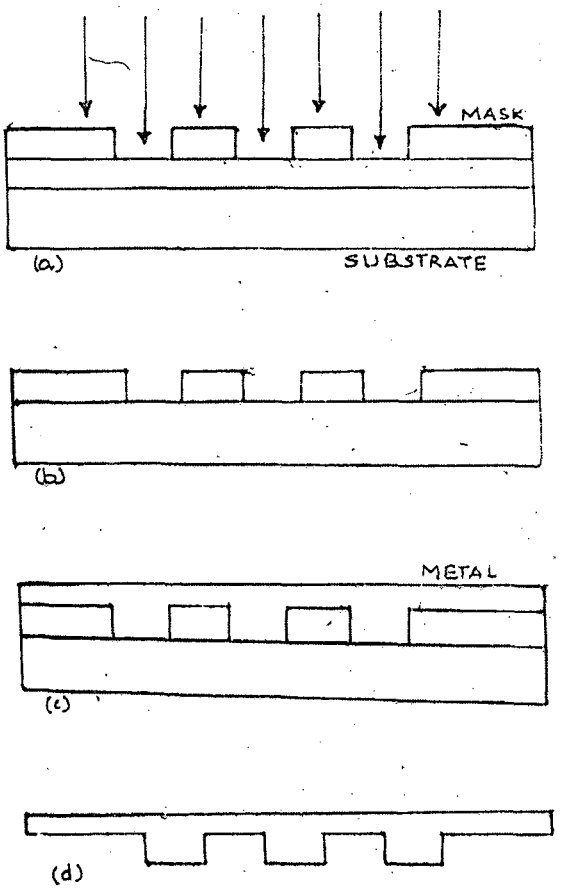
### Microstereolithography

Microstereolithography has a great potential for making chemical chips.It can build three dimensional structures, which opens up the possibility of direct ,rapid formation of complex three dimensional systems. A laser beam , or other focussed light source, is used to cure a material by writing a three - dimensional pattern. Usually, the material is a liquid , which is cured to form a solid,and needs to be photoinitiated before curing.Microstereolithography can produce micron & sub-micron features in three dimensions,which allows for many functions to be condensed onto a single chip.

**LIGA:** The LIGA process derives its name from a german acronym that translates as ' Lithography, electroplating and moulding'. This three stage process can be used to manufacture high aspect ratio, three dimensional microstructures in a wide variety of materials including polymers, metals, ceramics and glasses. The first step involves irradiating a polymer resist using laser light, electron or ion beams, or X-rays from a synchroton radiation source. X-rays are essential for producing deep and very high aspect ratio structures. All methods use a shadow printing process.

In the deep X-ray lithographic process, a two-dimensional absorer pattern from a mask is transferred into the depth of a thick resist by the chemical changes induced by a highly directional beam of X-rays. Developing the irradiated areas of the resist produces a 3D replication of the pattern. A metallic master mould can then be produced by electroplating into 'free' areas. This mould can also be used for injection moulding or as an embossing tool.

LIGA offers several advantages: it is relatively cheap, can achieve high precision and can be used on a wide range of materials.




(a) A mask is placed on top of the polymer resist and the structure is irradiated.

(b) The mask is then removed and the polymer is 'developed'

(c) The new structure is then electroplated.

(d) Finally the metal is separated from the polymer ready for use as a mould.

**Figure 3: LIGA process**

### Applications of Microfabricated Devices

1. **Microreactor :** Microfabrication offers many advantages. It not only reduces consumption of expensive reagents, fluidic components with small dead volumes, but also improves separation resulting from higher surface-to-volume ratios, integration of sensors and actuators, parallel screening and mass fabrication of multiple units by replication. Chemical engineers often use small reactors are faced with bench top analytical equipment and large panels of complex fluid handling manifolds. With the continual advances in μTAS and microfabricated reactors, these macroscopic test systems could eventually be replaced by PC-card sized microchemical systems consisting of integrated

microfluidic sensors, control and reaction components. Such systems would clearly require less space and utilities and produce less waste. They would enable high-throughput screening of catalysts and process chemistries under realistic conditions, which has proven difficult in current combinatorial approaches. Moreover, the small dimensions imply laminar flow, making it feasible to fully characterise heat and mass transfer and exact chemical kinetic parameters from sensor data.

Microreaction Technology is also expected to have an impact on chemical production. The high heat-transfer rates and mass-transfer rates possible in microfluidic systems could allow reactions to be performed under more aggressive conditions with higher yields than achievable with conventional reactors. More importantly, new reaction pathways deemed too difficult in conventional microscopic equipment e.g. direct fluorination of aromatic compounds, could be persued. Even if a microreactor failed, the small quantities of chemicals released accidentally could be easily contained. Moreover, the presence of integrated sensor and control units could allow the failed reactor to be isolated and replaced while parallel units continue to produce.

Dupont has synthesized a number of potentially hazardous chemicals, including isocyanates, in a microreactor formed by bonding Silicon wafers patterned to form channels, preheaters and catalytic reactor sections. The reduced consumption of expensive reagents, fast responsive time and actuators inherent in microfabricated systems are attractive particularly for screening of biological samples. Recent DNA detection units are essentially microchemical systems that combine reagent dosing, controlled reaction, separation and detection. For example, the integrated silicon and glass microfluidic device developed by Burns et al. meters reagents and DNA-containing solutions, mixes these solutions and then amplifies or digests DNA in a temperature controlled reaction chamber. The reaction products are subsequently separated by electrophoresis and detected by Fluorescence using an on-chip photodiode. Such devices have obvious applications in gene sequencing, medical diagnostics, and biohazard detection.

**Parameters :** Thin wall reactors offer the opportunity for integration of flow and temperature sensors on the external side. The micron-thick wall provides good thermal contact with the catalysts in the active reactor wall may be manipulated by adjusting the thickness of the wall and choosing material of different thermal conductivity. Thermal isolation is useful using the thin-wall reactor as a calorimeter, but also creates the potential for multiple steady states for lightly exothermic reactions. Increased heat conduction out of the catalyst removes the multiplicity and opens mild reaction conditions typically not accessible in conventional reactors. The integrated heaters and temperature sensors combined with the low thermal mass of the wall has the further advantage of fast thermal response times. The use of a permeable membrane, instead of a thin wall, allows the integration of separation with chemical reaction, as in microscopic membrane reactors. For example, the integration of a submicron thick palladium membrane makes a high efficiency hydrogen purification device and provides the potential for conducting hydrogenation and dehydrogenation reactions.

The small dimensions in microreactor channels guarantee laminar flow so that mixing occurs primarily by diffusion. These characteristics become both a challenge and an advantage for liquid-phase reaction systems. To accelerate mixing, most liquid-phase reaction systems rely on splitting and recombination of fluid streams several times so that a laminated fluid is created with an increased fluid interface and shortened diffusion path. Alternatively, the liquid feed can be introduced to produce a laminated stream. The choice of design becomes a trade-off between mixing speed, pressure drop, volume flow and feasibility of microfabrication. The relatively slow mixing phenomenon can be exploited in phase transfer reactions, as well as in novel microfabrication schemes.

The need to develop novel structures with controlled surface characteristics suggests that microreactor fabrication must go beyond classical micromachining and silicon MEMS techniques. Microfabrication in glass already forms the foundation for many biological devices because of the need for an insulating substrate for electrophoresis. Fabrication in plastic using embossing and injection moulding techniques is rapidly expanding. The family of chemical self-assembly and microfabrication techniques developoed by Xia and Whitesides called "Soft lithography" further provide unique opportunities for microfabrication and chemical tailoring of surfaces to particular application. Its strength includes the ability to transfer patterns onto nonpolar surfaces, formation microstructures and compatibility with a wide range of materials. These techniques have already produced unique microstructures and capabilities that could further advance microchemical systems.

To move beyond the laboratory into chemical production, microreactors must be integrated with

sensors and actuators either on the same chip or through hybrid integration schemes. It was the integrated circuit that created the Microelectronics and Computer revolution, not the transistor itself. The integration of chemical systems with sensitizers in TAS is already a rapidly expanding field, and cross-fertilisation with microreactors for chemical synthesis will result in integrated chemical processors. The packaging of multiple reactors presents significant challenges in fluid handling, and local reactor monitoring and control, which have not been previously addressed in traditional design of chemical plants. Thus the realisation of microreaction technology offers tremendous multidisciplinary research opportunities across biology, chemistry, materials and electronics, as well as in the traditional chemical engineering subdisciplines of catalysis, transport phenomena, reaction engineering and systems.

Over the past decade, many so called micrototal analytical systems (μTAS) have been reported in literature. Typically 2-3 $cm^2$, these devices contain a network of interconnecting channels (50-200 μm in cross-section) and are fabricated in plastic, silicon or glass predominantly by photolithography, hot embossing (for polymers), and injection moulding or laser abalation techniques. A common pumping technique is Electro-Osmotic Flow (EOF), which provides voltage controllable flow of small (ml) sample volumes with no mechanical moving parts. In addition to the EOF of total solution, the electric field in a small capillary also causes electrophoretic movement of charged reagent species, which neither retards nor accelerates species' mobility. These features of voltage control and species-selective mobility are experimentally realised in capillary electrophoresis columns for performing difficult separations. For systems that do not support EOF, including non-aqueous solvents required for organic synthesis, we could use alternative methods such as hydrodynamic pumping and electro-hydrodynamic pumping.

Let's take a particular example of synthesis of cyanobiphenyls using a modified Suzuki reaction to couple an aryl halide and organocarbon cyanobiphenyls using a continuous flow microreactor. The system uses EOF to mobilise the solvent THF, which, like many other organic solvents, exhibits very low natural EOF properties. It was possible to enhance flow rates to tens of μl $min^{-1}$ by preparing the solvent as a 75:25 (by volume) mixture with water and incorporating a microporous silicate structure within the flow channels. In addition to acting as pumping mechanism, the microporous silicate was also used to immobilize the Palladium catalyst .

By using the continuous flow microreactor shown in Fig.2 , it was possible to control the sequential delivery of reagents to the catalytical surface in a particular manner which gives a higher yield and better selectivity than a bulk reactor.

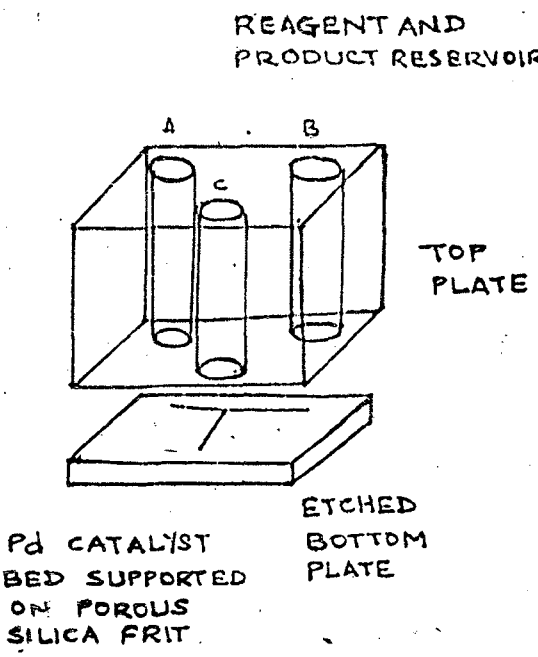



**Figure 4: Continuous flow organic synthesis microreactor**

The procedure involved initially leading 100 μl each of phenylboronic acid (0.1 M) and 4-Bromobenzonitrile (0.1 M) both in 75% THF aq. into reservoirs. A and B respectively with reservoir C being filled with only 30μl of the same solvent. Pt electrodes were placed in each reservoirs to generate required EOF. Electrode voltages were adjusted to give a continuous flow of phenylboronic acid from reservoir B (+200 V) to C(ground) into which scientist periodically injected timed slugs (of 5s duration) of 4-Bromobenzonitrile by applying ±200 V to reservoir A relative to reservoir C (ground). The total volume of Cyanobiphenyl product solution in reservoir C at the end of each reaction sequence and determined the yield. By reducing the system size by a factor of 10 reduces reagent consumption by a factor of 1000 and increases mass transport by diffusion by 100 fold. Although the synthesis of cyanobiphenyl itself is not novel, the control and selectivity offered by the microreactor could be the route to new and exciting characteristics. In particular microreactors offer more significant advantages for reactions that are oxygen-,light-, or moisture-sensitive and where

protection from a potential **explosive** or toxic source may compromise safety. In addition, the opportunity to perform atom efficient and/or nonlinear reactions in which we can isolate intermediates or products at will , offers scope for exciting and chemistry to be explored in the hand of imaginative.

**2. Microchips to deliver reagent at an electronic command[3]:** Chemical Enginner Prof. Robert S. Langer , graduate student John Santini Jr & Material Science Prof. Micheal J Sima[11] from MIT have reported that they could construct a microchip that can release fluorescent dyes & radiolabelled compounds in a controlled manner , on giving an electric command.

**Process:** The prototype microchips contain tiny reservoirs with different solid, liquid or gel materials inside. When an electric potential is applied to an electrode, a thin gold membrane over a reservoir imat dissolves allowing the materials inside to come out. The MIT chip makes it possible to control the rate of release & also the exact time of release of each component in a set of reagents.

**Parameters:** The chip measures 17 mm on a side, contains reservoirs of about 25-µl capacity each. About 1000 resevoirs can be accommodated on the chip. The designers laid stress on the fact that it should avoid moving parts which could break or get stuck up. Instead they hit upon the mechanism of Electrochemical dissolution of membranes.

**Advantages:** The chips could be implanted subcutaenously and used to deliver potent drugs, such as hormones , steroids or painkillers. In case of patch delivery, this can be integrated with transdermal devices. They can be swallowed along with pills that could be programmed to release different materials in the gastrointestinal tract. A number of diagnostic applications is also anticipated. They could compliment microfluidics, microreaction and DNA Chip technologies. The reagent release can be made possible through remote-control, pre-programming and biofeedback.

**3. Microfluidics - mixing nanoliters in microseconds:[4]** Researchers at the Princaton University have developed a tiny mixing vessel in which a submerged fluid jet is used to achieve fluid mixing times of less than 10 microseconds. Mixing, being diffusion driven , is normally a slow process on microscopic scales but can be sped up by introducing turbulence. Turbulence is difficult to predict & control, so the researchers looked to speed up mixing by shrinking the size scale.

**Process:** The device consists of two channels intersecting at right angles & open to viewing through a conership from above one fluid to be mixed flows under pressure through one channel. The second fluid enters the second channel also under pressure , from both ends & converges at the intersection where it hydrodynamically focuses the first stream down to a size at which diffusion across it takes mere microseconds. The mixed sample is delivered in a controlled laminar flow at rates of nanolitres per second.

**4. Microchip identifies pathogens rapidly[5]:** Cheng[7] & his colleagues at the Biochemical chemical company Nanogen in San Diego have developed a penny-sized microchip that extracts bacteria from blood samples using electricity. Current laboratory tests take days to identify the bacterium causing an infection. Biotechnologists are trying to develop lab-on-a-chip concept so that small silvers of Si can analyse blood or saliva in few minutes. The main challenge in this type of devices is to isolate pathogens, which are small in number compared to the millions of human cells.

Cheng & colleagues have successfully tested a square cm array of electrodes that can be programmed to attract only the cells wanted, rapidly separating them from the blood. A given cell under the influence of the current is charged positively or negatively depending on its electrical properties. In their trials , Cheng tried a frequency by trial & error that gave the bacteria negative & the human cells a positive charge.

After zapping a sample with blood spiked with E.coli , Cheng switched the array to direct current. The bacteria struck to the new positive electrode while the blood cells are repelled. Then the bacteria were ruptured with a 400 V shock. This short -circuits the cell membranes & proteins, he analysed the purified genetic material on a second electronic chip for the presence of bacterial DNA . The entire procedure is claimed to be over in 30 min. So far four types of bacteria have been separated including *Streptococcus* & *Staphylococcus*. The chip has also cultured carcinoma cells & thus may be useful in diagnosing cancer.

**5. Revolution on a square centimetre:** Bioelectronic chips may offer a convenient & relatively hands off method for isolating, lysing & detecting micro-organisms in complex samples. Heller's $1cm^2$ bioelectronic chip consists of an addressable array of 25 platinum 80 micrometer diameter microelectrodes covered by an agarose permeation layer is a 4.84 microlitre flow cell. The Si chip is

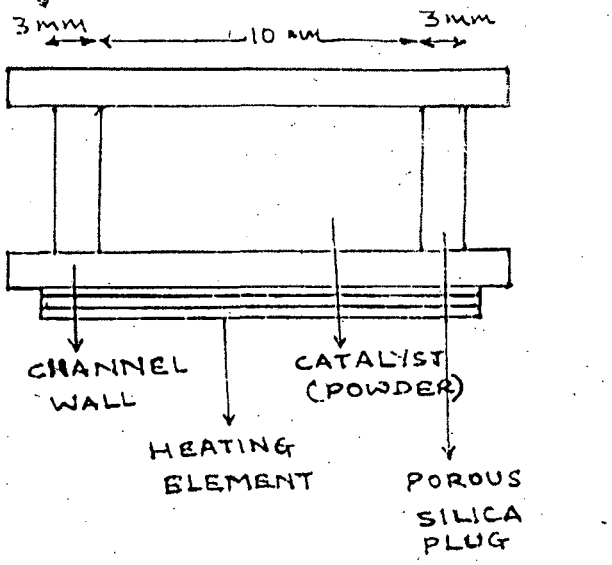
manufactured using the same type of photolithographic & deposition techniques employed in the microelectronics industry. The alternating current field that is set up in the chip separates cells by electrophoresis. Different cell types locate to different regions of the microelectrode array. Weakly held cells located in interelectrode regions of low field strength can be selectively removed by washing. The chip isolates E.coli cells from a whole blood sample in about 4 min: On chip lysis of the E.coli isolated out the microelectrode array is achieved by a series of 500 V pulses followed by incubation with proteinase K to digest proteins. The lysate is then transferred to a second bioelectronic chip for an electronically enhanced hybridisation assay. This bioelectronic chip is one example of a new wave of microminiature analytical devices under development for applications in drug discovery, genetic testing & separation science. The size of the chips varies, but they are typically metre sized structures within microlitres to nanolitre volume reaction chambers.

Considerable strides have been made in this type of integration. Chips with heaters, valves, pumps, microfluidic controllers & electrochemical & Electro-luminescent detectors have been produced. Also chips now exist that combine a range of sample preparation, analysis & detection reactions.

6. Another group of researchers led by Wolfgang Ehrfeld[12] at the Institut fur Mikroteknik in Germany, has demonstrated how microreactors can be used for hazardous reactions. They produced Hydrogen Cyanide on a microreactoe via Andrussow reaction route. The heart of the reactor was a heated catalyst made up of an array of 60µm channels. The gas was rapidly heated before entering the reactor microchannels, and after the reaction, a micro-heat exchanger rapidly cooled the products before undesirable side reactions could occur, such as the hydrolysis of HCN to ammonia. This again illustrates the benefits of the high rate of heat exchange and mass transfer possible in microreactor. This group has also shown that direct fluorination of aromatic compounds is feasible using microreactors. For conventional scale synthesis , a single step route is not normally possible because of Fluorine's low solubility and the highly exothermic nature of the reaction. Instead the reactio needs to be either multistep or performed at very low temperature, and the conversion efficiency is very low because of efficient temperature management, the reaction is possible.

7. Reactor chips are not necessarily simple devices. Indeeed Orchid Biocomputer in Princeton, US, has produced a complex multilayer chip where fluid flow is controlled by suface tension forces. The device can carry out multistep synthesis of over 100 compounds simultaneously. Albert van den Nen Berg and co-workers at the MESA Research Institute at Univeersity of Twente, the Netherlands have produced a silicon tube inside a second outer silicon tube. The inner tube was made *in situ* by a combination of etching methods, and resulted in a tube-in-a-tube channel that has great potential for Microdialysis or counterflow heat exchangers.

8. Catalysts represents an important extension of microreactors, and many groups have used catalyst in microreactors for synthetic applications. Reactions investigated include partial oxidation of methane to syn gas, demonstrated by workers at Forchungszentrum Karlsruhe[14] and Institute fur Angewandte Chemie Berlin-Adlershof, and the dehydration of alcohols to the associated alkene, which we have performed at the University of Hull. This later wok used a silica microstructure made *in situ* to retain the powdered catalyst in microchannel as shown in figure below.




**The catalyst packed within the microreactor is retained by an *in situ* fabricated frit.**

**Figure 5: Getting a catalyst in microreactor**

This support is also suitable for immoboilisation of enzymes, so it could be used as a microreactor for enzyme-mediated reactions.

9. The efficiency of a microfabricated devices for the rapid mixing of two liquid streams has been demonstrated by observing the rate of fluorescence quenching. The device was made from a 5 mm by 10 mm glass/silicon/glass sandwitch. Channels were etched into both sides of the silicon wafer. The 16 channels on the front split the first liquid into 16 separate streams, while the channel on the reverse

face split the second liquid into 16 streams. When they are mearged, mixing occurs rapidly. 95% mixing is achieved in 15 milliseconds.This occurs because the thickness of the liquid streams is reduced into n individual laminates, which makes mixing $n^2$ times faster . The glass layers provided inlet holes for the liquid streams and were attached to the Si wafer by anodic bonding. The fluorescence quenching was followed with a camera mounted onto a microscope or a photon multiplier tube for quantitative measurements. Overall, it was shown that in situations where rapid mixing is desirable, a mixing device such as this would prove valuable. Typically applications for such a device includes syntheses where the reactants need to be completely mixed.

10. Khandurina[10] & Co-workers have used a porous silicate layer in a microfabricated device for sample preconcentration & electrophoretic analysis.The porous membrane chip as shown in the following figure, was made from a glass substrate by photolithography & wet chemical etching using hydrofluoric acid/ ammonium fluoride.

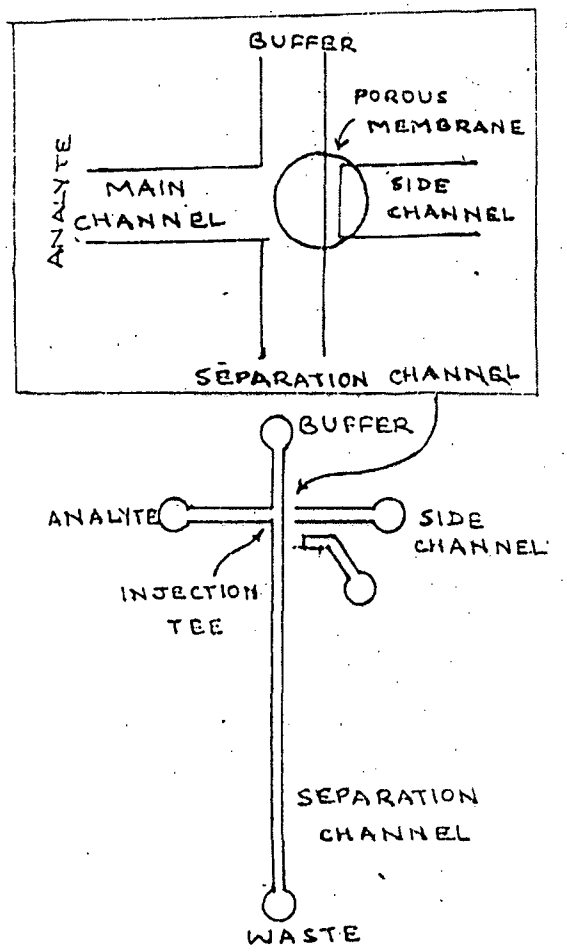



**Figure 6: The microchip for DNA concentration and separation, with the porous membrane region enlarged**

The top plate was attached by a low temperature bonding process where potassium silicate solution is spin coated onto the substrarte before bringing the surface into contact with the substrate. The porous membrane was then made by polycondensation of silicate film. The device has been applied to DNA preconcentration as, when a voltage is applied from the analyte reservoir to the side channel, only small ionic species can cross the membrane. The overall result is an appreciable concentration build up of DNA in the channel adjacent to the membrane. Once this step is completed, electrophoretic separation can be used in the separation can be used in the separation channel by applying a 1kV potential. Typical channel sizes were 8-10 micrometer deep and 60-65 micrometer wide at half depth ; the porous memnbrane varied from 3-12 micrometer in thickness. The separated DNA fragments were detracted by laser-induced fluoroscence monitored by a charge coupled device or a photon multiplier tube. By carefully controlling the applied potentials, no bleed of DNA from the analyte channel was observed during separation. When the porous membrane was incorporated onto an analysis chip, the DNA was concentrated by two orders of magnitude before separation.

11. Tan[8] and co-workers have developed in-tube solid-phase microextraction for the analysis of organic solutes in water. Theuy used 1m lengths of convebntional Gas-chromatography columns to extract components such as Toluene, Benzene, Xylenes and Phenols from water. This was achieved by first passing an appropriate quantity of substance typically 1-2ml through the capillary using Nitrogen pressure. See following figure.

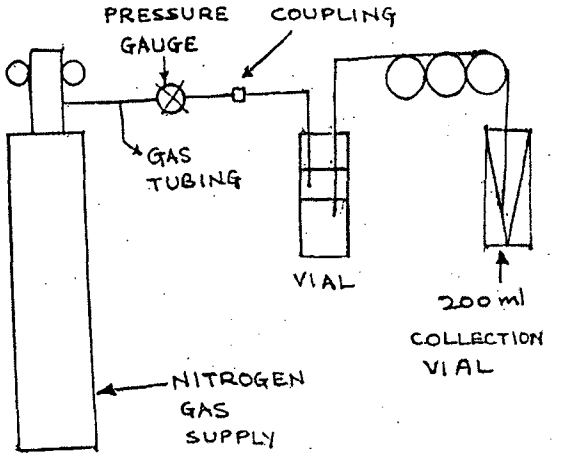



**Figure 7:**

Once the sample has passed through the column , the absorbed material can be removed by passing a small volume of stripping solvent through

the column. the collected extract can than be analysed by GC. The range of polarities available for extraction face in conjugation with reusable capillaries makes this a useful technique. The future logical development is to use thermal desorption to remove the adsorbed organics and introduce them on-line to the GC instrument.

**12. Capillary Electrophoresis on Chips :**[15] Capillaries have been formed more requently by wet-etching rectangular channels approximately 20 x 100 micrometer into an inorganic substrate and covering them with a transperant plate. Recently, channels have been molded and cast into polymers in an effort to reduce the fabrication cost. But the spectral properties of polymers frequently limit the detection. Fortunately, CE (Capillary Electrophoresis) separations in rectangular channels on chips are identical to those in open-tubular capillaries and so the considerable scientific literaure on CE in open-tubular capillaries applies to chips. If a separation has been demonstrated in a fused-silica capillary, similar or identical behaviour can be expected on a chip.One disadvantage of CE on chips is that the column length does not generally exceeds 5cm unless techniques such as synchronized cyclic capiliary electrophoresis are used.

Detection is generally achieved by laser-induced fluoroscence or absorbance with a pathlength of 20 micrometers or less. A tenfold increase in sensitivity was obtained by increasing the pathlength of the incident light with a 140 micrometer detection cell etched into the wafer at a 90 degree angle to the separation and exit channels. (In this detection-cell configuration, light is introduced parallel to the channel axis as opposed to crossing the channel width.) This flow cell was illuminated with optical fibres inserted horizontally into the chip through non-fluidic channels, aligning the fibres with windows at both ends of the flow cell.

The major issue with CE on chips is not so much what types of separations are possible but what does, the CE-on-a-chip format enable? In which circumstances does chip-based CE provide a distinct advantage over open-tubular fused-silica CE systems? One advantage is the ease with which pre- and post-separation chemical reactions may be integrated into the analytical process. Another is the ease and the speed of sample introduction; the cross-typed inlet for CE-on-chips has been a major advance, enabling samples to be aliquoted in less than 1 second. Yet another is the ability to use very short separation pathlengths : very short open-tubular capillaries are difficult to operate but combining the advantages of the cross-type inlet and very short columns, it has been possible to carry out CE separations in seconds. Perhaps the greatest advantage of CE on chips is the ease with which parallel processing systems may be constructed.

**13. Capillary Electrochromatography on Chips:**[15] When electo-osmotic flow (EOF) is used to transport the mobile phase in liquid chromatography, it is known as capillary electrochromatography (CEC). CEC is very similar to HPLC except that , in CEC , the mobile phase is driven by voltage alone and so there may be an electrophoretic-mobility component to separations, and that the flow profile in CEC produces less band spreading than in HPLC. CEC on a chip is most easily executed in an open-channel format ; that is , the stationary phase is immobilised on the channel walls. Using channels with a croass section of 10 x 100 micrometers, CEC separations have been achieved in few minutes. Although these remarkable seprations will be adequate in many applications, they probably do not represent the limits of resolutions and speed on chips reducing the channel width to less than 2 micrometers should increase the resolution. A closer approximations of a packed bed chromatography column has been achieved by using in situ micromachining to fabricate micrometer sized particle like support structures for liquid chromatography columns in a single quartz wafer.

Major advantages of these columns are that

1. Supports fabricated in situ are attached to the column walls.
2. The flow channels are very uniform in size.
3. The channel dimensions are independent of monolith size.
4. There are no unswept lateral channels.
5. Mechanical packing procedures are eliminated.
6. Intercolumn variability is reduced.

All fluidic components of the system are fabricated in situ including the solvent reservoir , filters and mixers , the mobile phase distributor, the support particles and the fluoroscence flow cell.

**14. High-Performance Liquid Chromatography on chips:**[15] Recognising that the flow rate from pressure-driven microbore HPLC columns is higher than the ideal for electrospray mass spectrometry, flow rates are being reduced to <= 50 microlitre per minute by decreasing the column diameter to <= 100 micrometer and the column volume to <= 1 microliter. The rectangular channels on chips have been slurry

packed with sorbents using a microfabricated column terminator to retain the particles. Sub microleter volume HPLC columns are possible in the array . Because these columns are more like a thin layer chromatography plate than a conventional cylindrical HPLC column, the volume is regulated by the column width.

As with all new technologies, there are surprises. One is that micromachining appears to be a more robust technology for producing columns than conventional technology. It is relatively easy to fabricate large numbers of columns reproducibly with millions of collocated monolith support structures. Some of the problems are equally surprising - It has been difficult to device a sealing system that

1. It allows the mobile phase to be brought into chips at upto 3000 psi and
2. it tolerates multiple coupling and uncoupling operations without leaking.

Increasing the path lenght to enhance the sensitivity of absorbance and fluoroscence must also be addressed. The need of Electrospray from multiple columns on a chip is yet another issue that needs attention.

**Drawbacks** Microminiaturisation is not without problems. however. The sub-microlitre sample volumes challenge the sensitivity of the current detection techniques. Evaporation must be controlled, strategies must be developed to solve sampling for low-abundance cells & user-friendly interfaces between the operator & microchip must be perfected.

**Conclusion:** The ultimate goal of microfluidics is to integrate as many different chemical processes as necessary to solve the given measurement problem on a single device. Lab-on-a-chip have progressed rapidly, but there are some obstacles that must be overcome to achieve the full power that many envision. Finally, Computer-Aided Design (CAD) tools that include fluidics, chemistry and biochemistry will be necessary to rapidly design the future generations of fluidics, microchips, much as microelectronic chips are designed today. Indeed the future for microfabricated fluidics devices - or the lab-on-a-chip looks quite promising.

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