DNA ANALYSIS SYSTEMS ON A CHIP

C. H. Mastrangelo

Center for Integrated Sensors and Circuits, Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109-2122, USA

This paper presents a review of microfabricated systems for genetic diagnostics. Genetic diagnostics are powerful technology drivers and excellent candidate applications for miniaturization technologies because the demand for inexpensive genetic information is essentially unlimited, and the cost and time for the diagnostic decreases with sample volume. Processing of DNA molecules in the microscale hence requires the implementation of microfluidic devices capable of handling, mixing, thermal cycling, separating, and detecting nano and pico liter liquid samples. The paper discusses some of the fundamental macroscale protocols used for genetic analyses and how these processes scale down to microscopic volumes. The construction and performance of microfluidic devices for DNA amplification, separation, hybridization, and detection are discussed showing that so far no fundamental impediments exist for MEMS-based genetic diagnostics.

1. INTRODUCTION

A fundamental requirement for the commercial success of any microfabrication technology is an application with a very large demand. These applications are essential technology drivers that provide sufficient economic pull for the adequate recovery of facility costs that sustain continued research into new and improved devices at very low unit cost. This paper discusses a new type of application for micro electromechanical systems (MEMS) that not only satisfies this requirement but also promises enormous potential for growth.

Genetic tests (or assays) have an enormous scope of applications in biotechnology and medicine, ranging from agriculture and farming [1] to the detection of pathogens in foods [2] to genetic diagnostics on human subjects [3]. Currently about 400 diseases are diagnosable by molecular analysis of nucleic acids, and this number is increasing daily. Humans have approximately 100,000 genes that could be potentially tested for defects or the propensity for diseases. Essentially with the same procedure, the contents of every gene on any form of life could be examined. Such a broad base application may prove to be the ultimate technology driver of all time.

Recently, there has been much interest in the implementation of microfluidic devices for genetic assays. These devices are excellent candidates for miniaturization because: (a) the demand for genetic information is essentially unlimited hence determined only by the cost of information retrieval, (b) the performance and costs of genetic assays can be improved in the microscale, (c) the same microfabricated part can be used for many different assays by changing the nature of its reagents, not the device construction, and (d) genetic assays can benefit from the automation and control provided by miniature electronic devices. The implementation of these devices presents new and interesting technological challenges. Genetic information is contained in a long polymer of nucleic acid, typically in solution in a weakly saline water-based buffer. The extraction of genetic information involves a series of chemical manipulations of the sample requiring mixing with reagents, thermal cycling, labeling and fragmentation analysis using conventional molecular biology protocols. A miniaturized

device for genetic assays is hence a chemical reactor capable of performing some or all of these functions in microscale volumes, including the detection of the assay outcome.

2. THE NATURE OF GENETIC INFORMATION

Genetic information in humans is stored in the cell chromosomes. Each chromosome consists of long, compactly packed, supercoiled linear polymer strands of deoxyribonucleic acid (or DNA). The chromosome information is stored as a long string of DNA fragments grouped as genes, each expressing an identifiable function or characteristic of the organism. In humans, for example, each of the 46 chromosomes is $50 - 400 \times 10^6$ units long while the single chromosome in the *E. coli* bacteria is 4×10^6 units long.

The units of a single DNA strand are called nucleotides. Each nucleotide consists of a base (B), a sugar linkage (S), and a phosphate bridge (P) as shown in Figure 1. The sugar linkage gives the nucleotide direc-

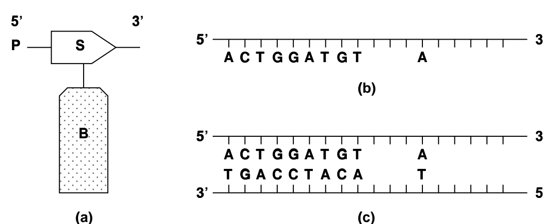


Figure 1: (a) DNA nucleotide, (b) single stranded DNA chain, and (c) double stranded DNA with complementary base pairs (bp). The two strands are joined by hydrogen bonds.

tionality with two distinct ends labeled $5'$ and $3'$. There are four types of nucleotides corresponding to four different bases: adenine, guanine, cytosine, and thymine, commonly labeled A, G, C, and T. Nucleotides can only be linked in a specific direction forming single strands of DNA as shown in Fig.1(b). Individual bases are hydrophobic, but strands of DNA are quite soluble in water due to the polar backbone. Single stranded DNA tends to attach (or hybridize) through weak hydrogen bonds to another strand of complementary base pairs (G-C and A-T) forming a double strand (or duplex). Double-stranded DNA is more stable in water because the hydrophobic bases are hidden by hydrophilic backbones.

DNA assays take two general forms. In diagnostic applications, the assay detects the presence of a specific base pair fragment in a fingerprint pattern matching fashion. In sequencing applications, the assay yields the actual base pair order. Sequencing assays inherently provide much more information than fingerprinting assays since test patterns are often not unique due to the presence of mutations. Both assays are performed using a set of well known molecular machinery. Typically, the analysis is performed over a small fragment of DNA whose concentration is chemically amplified using enzymatic techniques such as the polymerase chain reaction (PCR). After the amplification, these fragments are chemically cut into smaller ones and stained with a fluorescent dye. Information about the nature of the sample is obtained from the analysis of the fragment population using electrophoretic separations or hybridization techniques.

3. BENEFITS OF MINIATURIZATION

In macroscopic protocols, relatively large microliter volumes of DNA are handled in vials and manually loaded into desktop thermal cyclers and gels separators. These macroscopic systems are slow and expensive. For example, a typical sequencing protocol requires 6-8 hours to complete at a cost of several hundred dollars. Further, the high cost of these systems restricts these tests to laboratories.

Alternatively, smaller sample volumes can be analyzed with miniaturized equipment. There is ample jus-

tification for the miniaturization of these systems in both clinical and research settings [4]. Scaling down the assays result in an increase in throughput due to reduced analysis times, reagent cost reductions due to miniscule reaction volumes, and much lower system costs due to introduction of batch fabrication techniques. Figure 2 shows the effects of scaling on the assay parameters for a cubic sample of linear dimension L/S with S as a scaling parameter. The volume of sample and cost of reagents scale by S^{-3}. The same scaling factor affects

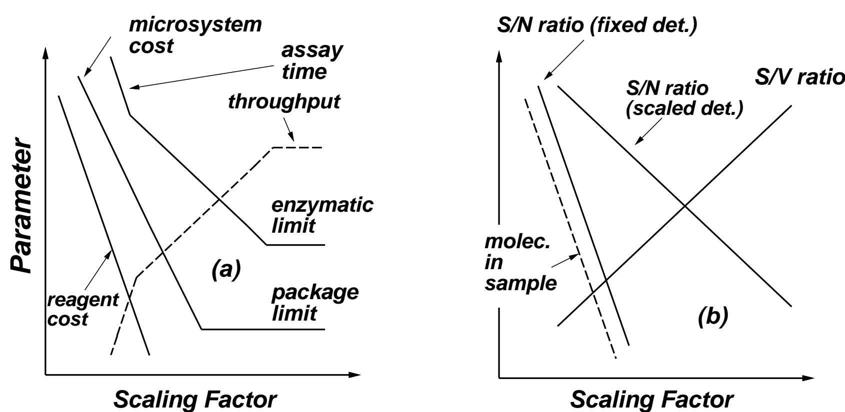


Figure 2: Scaling of assay parameters (a) costs and time are reduced. (b) The detector S/N ratio is degraded by scaling.

the thermal cycle time while separation time Scales by S^{-1} (a much celebrated argument [5]). Ultimately, the minimum assay time is limited by the speed of the enzyme itself (30-100 bp/s). Microsystem cost scales by S^{-2}, but it is limited by the package cost.

The benefits of scaling come at the expense of stretching detection limits. For a fixed concentration, the number of molecules in the sample scales by S^{-3}. If the detector area is fixed, the signal-to-noise ratio (S/N) is severely degraded by S^{-3}. The S/N reduction is not as severe if the detector area scales with the sample (S/N $\propto S^{-1}$). This favors the use of miniaturized detectors placed close to the sample.

Scaling also increases the surface-to-volume ratio of the sample (S/V) accentuating the influence of surface phenomena such as enzyme-wall adsorption and sample evaporation that may affect the microsystem performance. Further, scaling causes hydrodynamic problems. The resistance of capillaries scales by S hence sample transfer requires high pressures. Capillary forces are also scaled by S making sample localization very difficult and the control of surface properties essential. It seems reasonable to assume that the scaling factor is determined by detection noise. At a scale of 10 μm the number of DNA molecules (100-1000) may reach the practical detection limits. It is conceivable that scaling factors of 100 or larger are possible when handling picoliter samples. This results in 100-fold increases in throughput and similar decreases in cost.

4. MICROFABRICATED AMPLIFICATION DEVICES

A number of micromachined devices [6] have been developed to accomplish faster chemical amplification cycles by basically reducing or eliminating the large thermal mass present in macroscopic systems. The first of these devices was developed by M. A. Northrup [6] at LLNL. This device consists of a microwell cavity structure formed in a silicon substrate by anisotropic etching. The well bottom is a thin silicon nitride membrane with polysilicon heaters on the underside as shown in Figure 3. This type of structure is essentially the same used for many bulk micromachined pressure sensors; hence it can be fabricated cheaply. The well lead is a glass slide bonded to the top. Due to its small thermal mass, this structure can be heated at rates of 15 °C/s and cycle times of about 1 min. A twenty cycle amplification in a 50 μL microwell was carried out roughly four

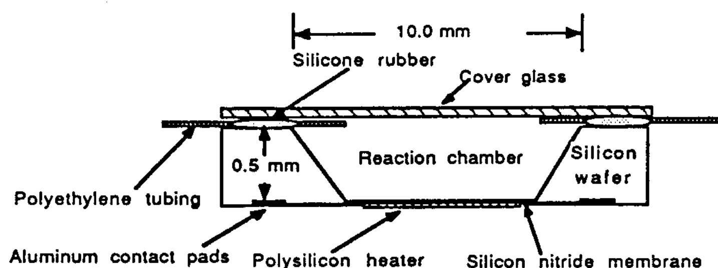


Figure 3 Silicon microwell for PCR reaction [6].

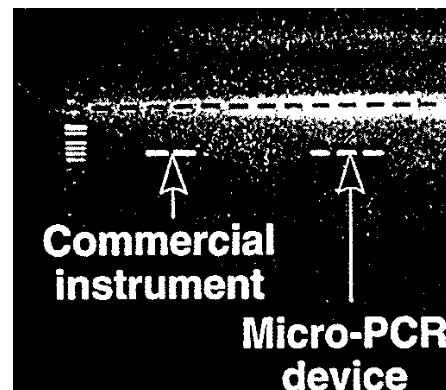


Figure 4 Comparison of DNA fragments from conventional and microwell PCR [6].

times faster than in a conventional cycler with a much lower power budget.

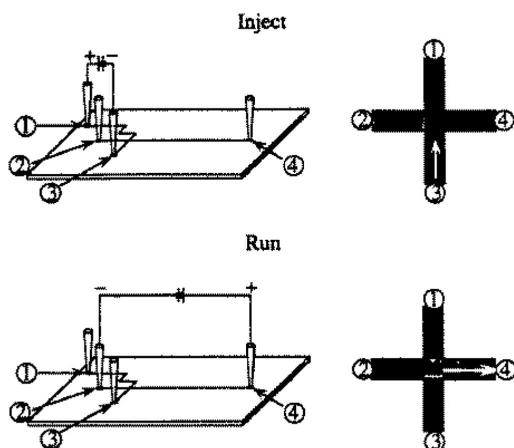
The microwell approach is suitable for array detection of multiple pathogens. Arrays of 8 and 24 wells with and without heaters have been fabricated on silicon anodically bonded to glass [7]. Reagents are loaded into each well using inkjet-type methods to provide capability of in-situ fluorescence. One of the major problems with the silicon-based devices is their cost. Simpler arrays of polypropylene microwells have been fabricated using conventional moulding and stamping methods with well depths of 250 μm. These passive devices require an external cooled CCD camera readout; therefore they must be used with an expensive reader. Recently, diode detectors have been used in silicon microwell structures to provide quantification of PCR products by electrochemiluminescence [8]. These devices show a detection limit of 40 femtomoles of DNA which is substantially less than that for commercial macroscopic devices.

5. ELECTROPHORESIS MICRODEVICES

In capillary electrophoresis (CE) systems, band resolution is not only determined by the separation voltage but also the length of the sample injection plug. High resolution separations with millimeter-sized plugs require long capillaries ($\approx$ 1 m) and separation times. Faster separation times could be achieved with shorter, microfabricated channels if used along with micro injection and more sensitive band detection schemes.

The feasibility, properties, and performance of electrophoresis devices microfabricated on planar glass substrates have been studied by many analytical chemists [9]. These devices consist of two crossing perpendicular channels. The first channel defines the sample injection plug and the second separates the sample. These channels are made by wet etching two 10 μm deep crossing grooves on Pyrex glass wafers [5]. Platinum electrodes are next deposited and patterned, and channels are next sealed with a top glass wafer with access holes bonded to the substrate thus forming two crossing capillaries. Due to the presence of the metal steps, hermetic bonding can be accomplished using cement or by thermally fusing the glass pieces at 660 °C. Other methods of fabrication for these devices have been recently developed [10].

Figure 5 shows a typical etch pattern configuration. The most important part of the device is the channel intersection which determines the size of the injection plug. After introduction of the mobile phase, the sample is placed in the lower reservoir. A low voltage is next applied across the vertical capillary forming a long plug that fills the capillary with sample (with no separation). Next the vertical capillary voltage is turned off and a high voltage is applied across the horizontal capillary moving the plug of sample at the intersection forward



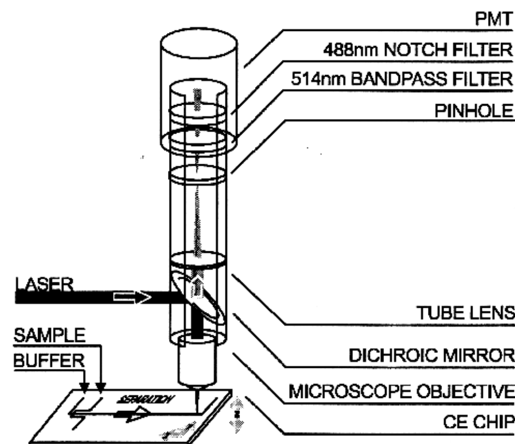
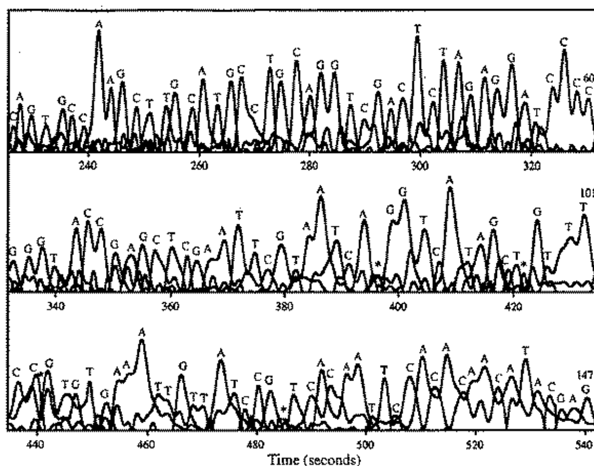


Figure 5 Simple cross-channel capillaries etched into glass are capable of fast, high resolution DNA separations [11].

Figure 6 Confocal epi-fluorescence readout for CE chips [12]

and resulting in a high resolution separation. Typically the migrating bands are recorded using a confocal fluorescence microscope focused at a specific spot [12] as shown in Figure 6. In the above scheme the injection plug length is enlarged due to lateral diffusion and convection eddies at the intersection [9]. The cross channel voltage configuration can be changed to form a sharper plug if counter currents are injected into the separation channel to prevent the plug diffusion [9]. The resulting plug volume is constant yielding higher resolution separations.

The performance of planar crossing channels for separations of DNA was demonstrated by Woolley [11]. Figure shows separations of DNA fragments on a short, $8 \times 50\ \mu m^2$, 3.5 cm long capillary. About 430 fragments were distinguishable in about 10 minutes separation time using fields of 200 V/cm. The chip separation is about 3-5 times faster than conventional CE and 50 times faster than slab gels. Direct sequencing has also been carried out in these structures using multicolor dyes.



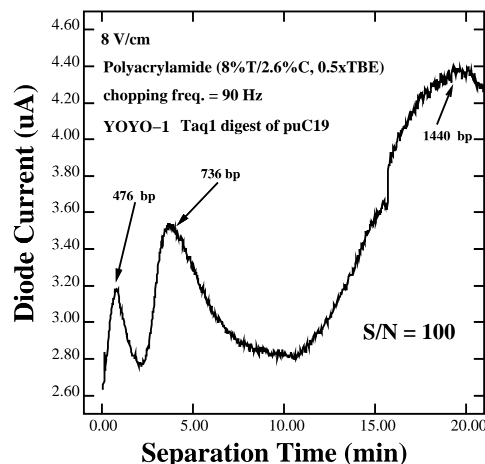


Figure 7 Separation of DNA fragments in planar glass chips using multicolor dyes and confocal microscopy detection [11].

Figure 8 Low-resolution separation of DNA fragments recorded with on-chip detector diodes thus eliminating the need for expensive readout optics [13].

The separation quality depends not only on the device geometry but also the type of separating medium. For DNA fragments, the polymerization uniformity of conventional slab gel matrix materials in capillaries such as polyacrylamide is difficult to control. For this reason, unpolymerized liquids such as linear acrylamide and hydroxyethylcellulose (HEC) are often used [14]. Unlike cross-linked polymers, these sieving materials can be pumped out of the channel allowing the reuse of the chip. In all of these devices, the presence of DNA bands was observed using fluorescence microscopy techniques. Therefore these devices must be used in a laboratory setting.

Recently, separation channels have been fabricated on silicon substrates with on chip detectors and filters [13]. DNA separations are possible on silicon substrates because high voltage drops which may breakdown the isolation dielectrics [15] are generally not required and when needed can be properly handled using very thick dielectrics. The presence of individual bands has been observed with fairly high signal to noise ratios (of about 100) as shown in Figure 8. The fabrication of these devices is discussed in section 6. On-chip detectors eliminate the need of expensive readout optics and open the road to low-cost disposable devices.

5. MICROMACHINED HYBRIDIZATION ARRAYS

Hybridization is the term used for the hydrogen bonding of two complementary single strands of DNA thus forming a double strand. This renaturation process of DNA occurs at specific temperature and salinity conditions. In hybridization-based DNA analyses, one of the strands is known (a DNA probe) and the other unknown. The hybridization bond is specific since it occurs only when there is a match of complementary strands. The presence of a double strand in the mixture (detected by fluorescence) is indicative of a match; hence hybridization serves is a sequence detection mechanism. There are many different kinds of hybridization, but the most relevant to this paper uses DNA probes which are immobilized. These probes are attached to a rigid surface using a linker molecule as shown in Figure 9. The DNA probes can be either synthetic oligonucleotides or longer DNA fragments typically arranged in array form.

Hybridization array devices can be micromachined using lithographic techniques [16]. Fodor's technique [16] permits the photo induced solid synthesis of oligonucleotides. First, a glass substrate is coated with a linker molecule that has a photochemical removable protecting group [17]. Upon the illumination of UV light, the protecting group is removed at selected areas. Next the first nucleoside with a photo-labile attached to its $5'$ end is placed on the substrate, bonding only to the deprotected linkers. The cycle is repeated to build oligonucleotides of arbitrary length one base at a time. A similar technique that uses protecting photoresist has been reported to produce smaller, 8 μm pixels [18].

The array probe patches range from 50-200 μm each; therefore a 1 cm^2 can contain 10-40,000 different probes. The number of possible oligonucleotides is much larger yielding, for example for a 15-mer probe, $4^{15} \approx 10^9$ combinations. Dense arrays with 20,000-96,600 probe patches have been reported [19]. Figure 10 shows the example fluorescent array signature of a test for the HIV virus. These type of devices are now widely used in genetic research [20]. Currently, the fluorescent signal for these devices is read using confocal fluorescence microscopes.

One of the important factors in these devices is the detection time. The hybridization of the sample to the probes is a diffusion controlled phenomena; hence slow. After the sample is poured on the array, it is typically necessary to wait for 90 minutes for the hybridization to complete. Recently, the hybridization speed was enhanced by the use of electric fields that direct the sample to the probes. Sosnowski [22] fabricated a platinum electrode array device on a silicon substrate passivated with silicon nitride and a permeable agarose

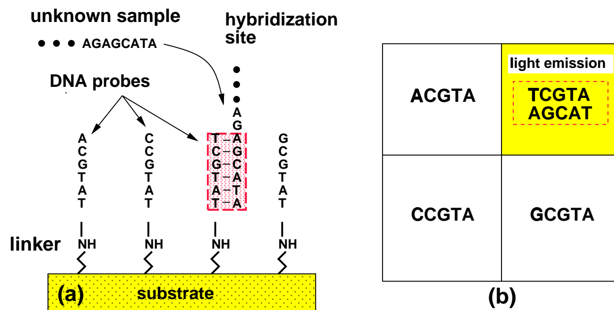


Figure 9 (a) Example immobilized oligonucleotide probes showing hybridization of unknown to specific probe. (b) Probes are arranged as planar arrays. The hybridized regions can be detected by the fluorescence of the duplex.

Figure 10 Fluorescent signature of hybridization array for HIV virus assay [21]. The pixels are 20-50 μm wide.

gel. Through the application of an electric field between the electrode and the sample, the hybridization time was reduced twenty five fold.

Further advances in this area will require improvements in inexpensive fluorescence detection. Some attempts have been made for simpler, less expensive detection techniques. Recently, hybridization arrays with radioactive labels have been imaged by placing the array in close proximity to a much cheaper conventional CCD chip eliminating the expensive readout optics [23].

6. INTEGRATED DEVICES

The devices discussed above are good demonstration vehicles for feasibility of molecular assays in microscale environments, but their practical implementation for low-cost applications presents more problems.

Many of these devices are crude in construction and not manufacturable in their present forms. Further, DNA assays require a combination of these devices, but there is no unifying platform that supports them [24]. In addition, most of these devices require external optical readers which restrict their use to laboratory environments increasing the assay cost substantially. A solution to these problems is an inexpensive integrated fabrication technology that accommodates all the necessary fluid, thermal, and detection functions under a common substrate. Simple integrated devices have been fabricated by Woolley [24] and Burns [25]. Woolley's device combined a vertical PCR microwell with a planar glass chip CE stage to perform DNA amplification and separation functions in under 45 minutes.

Burns' device incorporated simple injectors, mixer, heating chamber, and separation channel with detectors. Diode detectors were constructed in a silicon substrate with a thick optical filter deposited on the surface that blocks UV excitation. Heaters and electrodes are next defined on the surface and passivated with silicon dioxide and p-xylylene. A network of capillaries and chambers is next formed above the surface by bonding an etched glass wafer on top of the silicon substrate. In this device, sample and reagents can be driven through the system pneumatically or by heated gas trapped in pockets behind the sample [26], and the sample spread is controlled through a series of hydrophobic stops [27]. This device demonstrated the motion, amplification, separation, and detection of DNA samples integrated in a single part. Integrated bulk glass devices are not manufactured easily because the surface of the detector substrate is not flat; hence hermetic bonding can only be

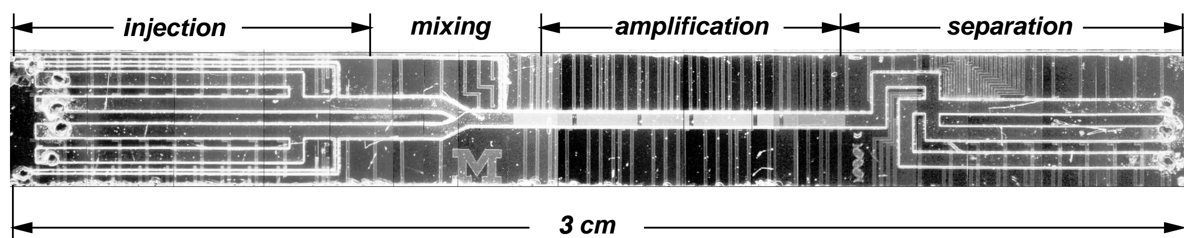


Figure 11: Example integrated glass device with injectors, mixers, amplification chamber, separation and detection [25]. The typical on-chip detector signal is shown in Figure 8.

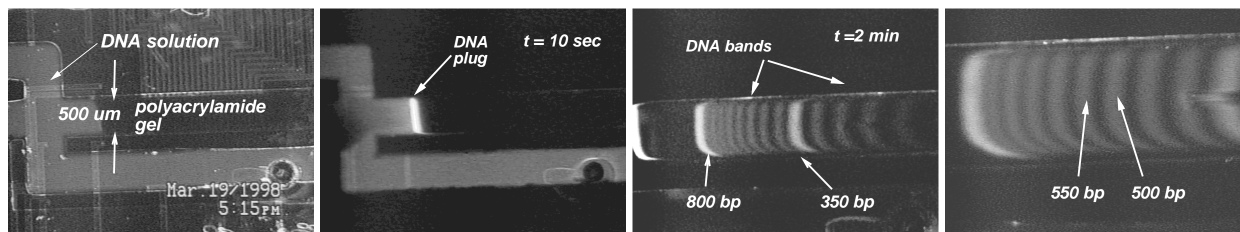
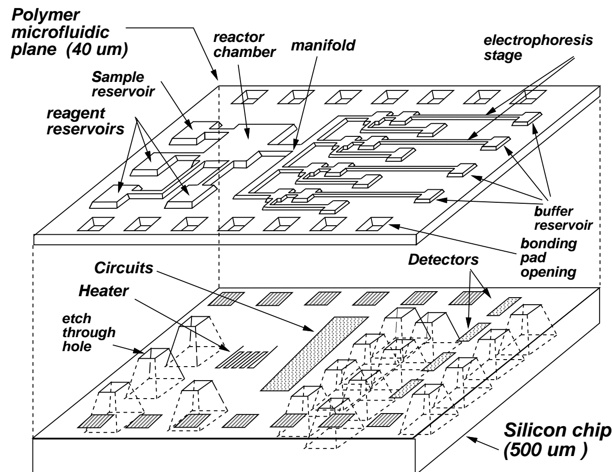


Figure 12: Injection and separation of DNA fragments on integrated device. The channel is $500 \times 50\ \mu m^2$. (50 bp ladder, 0.13 μg/μL, SYBR Green, 8 V/cm, 10%T:2.6%C polyacrylamide) [25].

achieved using epoxies. Further, heating protocols are very inefficient because of the high thermal conductivity of silicon.

Alternatively, these devices can be fabricated using flexible plastics [28–31]. These materials can be bonded or deposited on most substrates relatively easily [32]. Further, many plastic materials are biocompatible, transparent, and provide a high degree of chemical and thermal isolation. Thick layers of plastic can also provide the necessary dielectric isolation between the detector and CE electrodes. Plastic-on-silicon devices have been fabricated by several groups [28–30, 33]. These microfluidic systems are partitioned into two stacked levels of functionality as shown in Figure 13. These devices are fabricated using a simple three mask, low-temperature, IC-compatible process that allows the integration of microfluidic and circuit elements. The capillary structures are constructed using a thick layer of sacrificial photoresist covered with a vapor deposited plastic, p-xylylene [32], or combinations of p-xylylene with thick polyimides. After etching holes through the walls, the sacrificial resist is removed by immersion in acetone. The fluidic elements are conformal to the substrate thus overcoming the planarization required by bonded structures. The capillaries are also optically transparent and can range from 0.5 μm to 50 μm in height. Figure 14 shows a top photograph of plastic polyimide/p-xylylene capillaries constructed on top of a regular CMOS circuit substrate. Figure 15 shows the cross section of p-xylylene channels constructed on top of a silicon substrate. The top wall is 10-20 μm thick. The resulting channels have fairly large volumes, are optically transparent, are conformal to the substrate, and are perfectly hermetic. One of the major virtues of the plastic fabrication process is that it only uses weak chemicals and low temperatures ($< 150\ ^\circ$C); thus the fluidic plane can be constructed on top of any conventional circuit substrate without chemical attack or thermal degradation of the underlying circuits.

Several microfluidic devices have been fabricated using this technology. Webster [29] fabricated CE channels with integrated detectors [25] as shown in Figure 16. In integrated devices, a droplet of DNA sample must be mixed with reagents and transported throughout the system; therefore a suitable mechanism for the injection and propulsion of droplets is needed. In Anderson's processor [34], these functions are performed using a set of externally driven pneumatic valves; therefore this system requires an external pump or compressed air source. The internal generation of sample propulsion mechanisms is difficult due to the need for valves that require



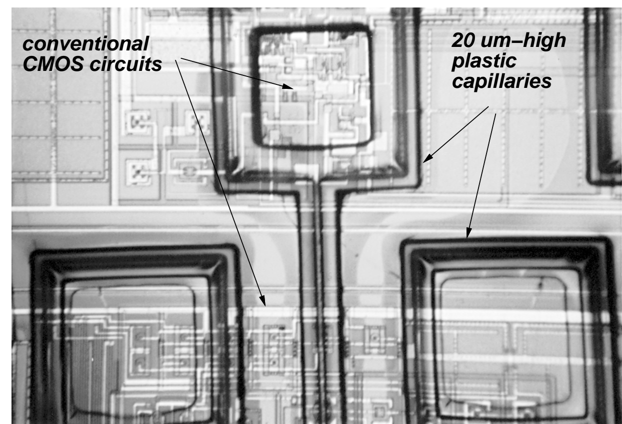
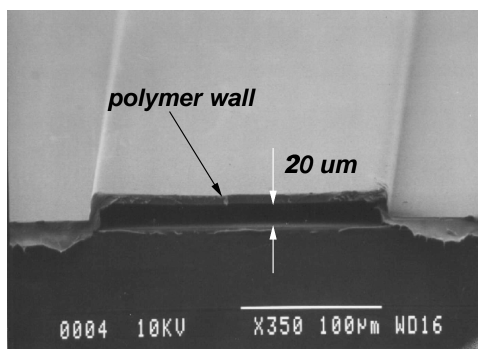


Figure 13 The polymer fluid channels and reservoirs are constructed by deposition of plastics on top of a silicon substrate. Etch-through holes are formed in the silicon substrate to serve as funnel-like inlets and reservoirs for the sample, reagents, and buffer solutions [28].

Figure 14 Top view of polyimide/p-xylylene channels constructed on top of a CMOS circuit. The low-temperature process does not degrade any of the circuit performance.



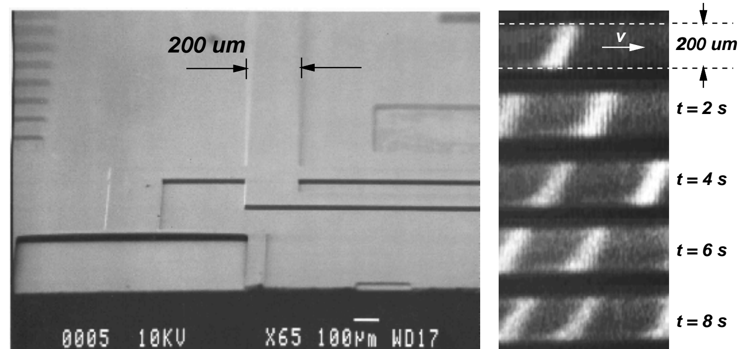


Figure 15 Example cross section of a p - xylylene polymer channel [28–30].

Figure 16 Capillary electrophoresis device fabricated using plastics on silicon. (a) The channel cross section is $200 \times 20\ \mu\mathrm{m}^2$. (b) Photograph showing separation of fluorescently labeled DNA fragments in plastic CE channel at elapsed times (HEC, 0.5%, 100 V/cm) [29].

moving parts. At the same time, the small dimensions of the capillaries make the pressure drops for inducing motion fairly large.

Recently, the motion of samples within these devices has been achieved taking advantage of the large capillary pressures present in these capillaries. Handique [27] used hydrophobic patches to stop the wetting of the solution, and a thermally expanding bubble to cut and propel individual sample drops. Man [30] developed capillary stops and injectors using a variant of the plastic process. The device uses a sharp neck in the channel that creates a surface-induced pressure barrier that stops the flow. The injector was driven by the pressure of an electrolytically generated oxygen bubble formed just behind the neck region. Electrolytic bubbles hold two important virtues. The bubble volume can be precisely metered since the integrated current is representative of the moles of gas. In addition, the power dissipation required for the bubble generation and drop motion is three orders of magnitude smaller than for the thermal drive. The injector shown in the picture only requires 100 μW of power to drive the sample forward.

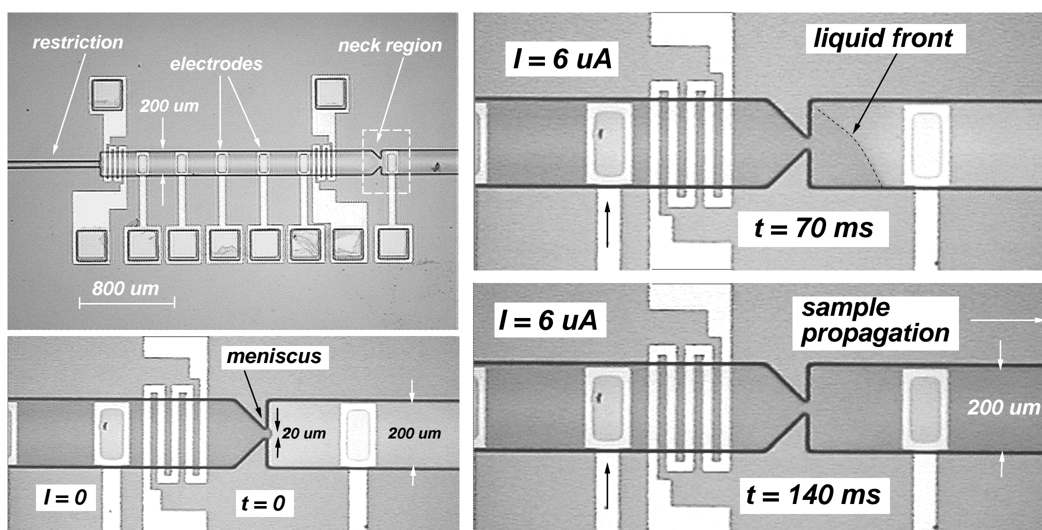


Figure 17: (a) Example stop valve/injector implemented using plastics on silicon. The sharp lateral constriction forms a pressure barrier that stops the flow. (b) The injector is activated by the formation of an oxygen bubble that overcomes the barrier re-establishing flow [30].

Unlike glass devices, plastic is more susceptible to the rapid evaporation of buffer and gels due to the larger solid-phase diffusion coefficients. Typically a combination of plastic capillary walls with glass coatings is necessary to eliminate these adverse effects.

7. PACKAGING AND INTERFACING

While much progress has been achieved toward the miniaturization of genetic assay devices, there are still fundamental challenges that must be resolved before the practical realization of these systems. Two important research areas that have not been addressed are chip packaging and interfacing.

Microscopic devices require both electrical and fluidic connections. The package must satisfy both of these functions. Electrical connections typically take the form of wire bonds while fluid connections require a reservoir or pocket for the introduction of the sample. From the packaging point of view, it makes sense to isolate these intrinsically different signal paths for several reasons. Electrical connections are fragile and do not need to be exposed hence they can be protected effectively using epoxies. On the other hand the fluid interface must be open for the introduction of liquid samples. A possible approach may use the backside of the chip for the fluid inlets while the front is used for the bonding wires. The fluidic interface is equally important and introduces additional problems. Sample loading in these devices is in general a difficult operation. A representative sample of 1 μL drop of DNA in solution requires a reservoir volume of 1 mm^3. This volume is fairly large compared to the total chip inner volume. Even at this larger volume, the introduction of sample in these small reservoirs requires good alignment and a very small dispensing device. These problems can be eliminated if an intermediate part that connects the macroscopic to microscopic scales is constructed.

Based on the above, two alternative interfaces could be feasible. A large reservoir with a sealable membrane could be incorporated within the package. This approach could permit the injection of relatively large 5-10 μL samples with a syringe. A second alternative could use direct wicking of the sample into the system. To date there is no literature available on practical packaging and interfacing schemes for electrophoretic devices.

8. CONCLUSION

The demand for genetic information is essentially unlimited, but conventional laboratory genetic assays are slow and expensive. Assay cost and time can be reduced by several orders of magnitude if the size of sample and analysis apparatus are reduced to microscale dimensions. The virtues and feasibility of micromachined devices for genetic assays have been recently demonstrated by many research groups. The enormous number of commercial applications has fueled very rapid technological advances in these devices spanning the fields of molecular biology, chemistry, and microfabrication.

References

[1] J. Buitkamp and J. T. Epplen, "Modern genome research and DNA diagnostics in domestic animals in the light of classical breeding techniques," *Electrophoresis*, vol. 17, pp. 1–11, 1996.

[2] P. Feng, "Impact of molecular biology on the detection of foodborne pathogens," *Molecular Biotechnology*, vol. 7, pp. 267–278, 1997.

[3] J. Reiss, "The polymerase chain reaction and its potential role in clinical diagnosis and research," *J. Internal. Medicine*, vol. 230, pp. 391–395, 1991.

[4] J. M. Ramsey, S. C. Jacobson, and M. R. Knapp, "Microfabricated chemical measurement systems," *Nature Medicine*, vol. 1, pp. 1093–1096, 1995.

[5] J. D. Harrison, K. Fluri, K. Seiler, Z. Fan, C. S. Effenhauser, and A. Manz, "Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip," *Science*, vol. 261, pp. 895–897, 1993.

[6] M. A. Northrup, M. T. Ching, R. M. White, and R. T. Watson, "DNA amplification with a microfabricated reaction chamber," in *Proc. 1993 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 924–926, 1993.

[7] M. Albin, R. Kowallis, E. Picozza, Y. Raysberg, C. Sloan, E. Winn-Deen, T. Woudenberg, and J. Zupfer, "Micromachining and microgenetics: where are they and where do they work together," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head' 96)*, pp. 253–257, 1996.

[8] Y.-T. Hsue, S. D. Collins, and R. L. Smith, "DNA quantification with an electrochemiluminescence microcell," in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 175–178, 1997.

[9] S. C. Jacobson, R. Hergenroder, L. B. Koutny, R. J. Warmack, and J. M. Ramsey, "Effects of injection schemes and column geometry on the performance of microchip electrophoresis devices," *Anal. Chem.*, vol. 66, pp. 1107–1113, 1994.

[10] V. L. Spiering, J. N. van der Moolen, G.-J. Burger, and A. van der Berg, "Novel microstructures and technologies applied in chemical analysis techniques," in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 511–514, 1997.

[11] A. T. Woolley and R. A. Mathies, "Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips," *Anal. Chem.*, vol. 67, pp. 3676–3680, 1995.

[12] T. Tang, G. Ocvirk, and D. J. Harrison, "Iso-thermal DNA reactions and assays in microfabricated capillary electrophoresis systems," in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 523–526, 1997.

[13] S. N. Brahmasandra, B. N. Johnson, J. R. Webster, D. T. Burke, C. H. Mastrangelo, and M. A. Burns, "On-chip DNA band detection in microfabricated separation systems," in *Submitted Proc. SPIE Microfluid. Dev. Sys.*, 1998.

[14] W. M. Sunada and H. W. Blanch, "Polymeric separation media for capillary electrophoresis of nucleic acids," *Electrophoresis*, vol. 18, pp. 2243–2254, 1997.

[15] D. J. Harrison, A. Manz, and P. G. Glavina, "Electroosmotic pumping within a chemical sensor system integrated on silicon," in *Proc. 1991 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 792–795, 1991.

[16] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lua, and D. Solas, “Light-directed spatially addressable parallel chemical synthesis,” *Science*, vol. 251, pp. 767–773, 1991.

[17] A. C. Pease, D. Solas, E. J. Sullivan, M. T. Cronin, C. P. Holmes, and S. P. A. Fodor, “Light-generated oligonucleotide arrays for rapid DNA sequence analysis,” *Proc. Natl. Acad. Sci. USA*, vol. 91, pp. 5022–5026, 1991.

[18] G. McGall, J. Labadie, P. Brock, G. Wallraff, T. Nguyen, and W. Hinsberg, “Light-directed synthesis of high-density oligonucleotide arrays using semiconductor photoresists,” *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 13555–13560, 1996.

[19] G. H. Hacia, L. C. Brody, M. S. Chee, S. P. A. Fodor, and F. S. Collins, “Detection of heterozygous mutations in BRCA1 using high-density oligonucletide arrays and two color fluorescence analysis,” *Nature Gen.*, vol. 14, pp. 441–447, 1996.

[20] G. Ramsay, “DNA chips: state-of-the art,” *Nature Biotech.*, vol. 16, pp. 40–44, 1998.

[21] R. J. Lipshutz, D. Morris, M. Chee, E. Hubbell, M. J. Kozal, N. Shah, N. Shen, R. Yang, and S. Fodor, “Using oligonucleotide probe arrays to access genetic diversity,” *Biotechniques*, vol. 19, pp. 442–447, 1995.

[22] R. G. Sosnowski, E. Tu, W. F. Butler, J. P. O’Connell, and M. J. Heller, “Rapid determination of single base mismatch mutations in DNA hybrids by direct electric field control,” *Proc. Natl. Acad. Sci. USA*, vol. 94, pp. 1119–1123, 1997.

[23] J. B. Lamture, K. L. Beattie, B. E. Burke, M. D. Eggers, D. J. Ehrlich, R. Fowler, M. A. Hollis, B. B. Kosicki, and R. K. Reich, “Direct detection of nucleic acid hybridization on the surface of a charge coupled device,” *Nucl. Acids. Res.*, vol. 22, pp. 2121–2125, 1994.

[24] A. T. Woolley, D. Hadley, P. Landre, A. J. deMello, R. A. Mathies, and M. A. Northrup, “Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device,” *Anal. Chem.*, vol. 68, pp. 4081–4086, 1996.

[25] M. A. Burns, C. H. Mastrangelo, T. S. Sammarco, P. F. Man, J. R. Webster, B. N. Johnson, B. Foerster, D. K. Jones, Y. Fields, A. R. Kaiser, and D. T. Burke, “Microfabricated structures for integrated DNA analysis,” *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 5556–5561, 1996.

[26] K. Handique, D. T. Burke, C. H. Mastrangelo, and M. A. Burns, “Nanoliter-volume discrete drop injection and pumping in microfabricated chemical analysis systems,” in *International Workshop on Solid-State Sensors and Actuators (Hilton Head’ 98)*, 1998.

[27] K. Handique, B. P. Gogoi, D. T. Burke, and C. H. M. anf M. A. Burns, “Microfluidic flow control using selective hydrophobic patterning,” in *Proc. SPIE Micromach. Conf.*, vol. 3224, pp. 185–199, 1997.

[28] P. F. Man, D. K. Jones, and C. H. Mastrangelo, “Microfluidic plastic capillaries on silicon substrates: a new inexpensive technology for bioanalysis chips,” in *International Workshop on Micro Electromechanical Systems (MEMS 97)*, pp. 311–316, 1997.

[29] J. R. Webster and C. H. Mastrangelo, “Large-volume integrated capillary electrophoresis stage fabricated using micromachining of plastics on silicon substrates,” in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 503–506, 1997.

[30] P. F. Man, C. H. Mastrangelo, M. A. Burns, and D. T. Burke, “Microfabricated capillarity-driven stop valve and sample injector,” in *International Conference on Micro Electromechanical Systems (MEMS 98)*, pp. 45–50, 1997.

[31] J. Hunt-Holmes, A. B. Frazier, and H. Swerdlow, “Integrated microchannels with metalic and polymeric channel sections,” in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 519–522, 1997.

[32] J. J. Licari, *Plastic Coatings for Electronics*. Krieger, 1981.

[33] L. J. Guerin, M. Bossel, M. Demierre, S. Calmes, and P. Renaud, “Simple and low cost fabrication of embedded microchannels by using a new thick-film photoplastic,” in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 1419–1422, 1997.

[34] R. C. Anderson, G. J. Bogdan, Z. Barniv, T. D. Dawes, J. Winkler, and K. Roy, "Microfluidic biochemical analysis system," in *Proc. 1997 IEEE Int. Conf. Solid-State Sens. Actuat.*, pp. 477–480, 1997.