Microfabricated Devices for Genetic Diagnostics

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Abstract—This paper presents a review of microfabricated devices 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. Genetic information is stored in long DNA molecules in solution. This information is processed and extracted using a series of enzymatic and other chemical reactions well known in molecular biology. 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. Some of the unresolved storage and packaging issues and future challenges for the practical implementation of these devices are also presented.

I. 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 of 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 [2–4] to genetic diagnostics on human subjects [5–14]. Currently about 400 diseases are diagnosable by molecular analysis of nucleic acids, and this number is increasing daily. Many of these assays were recently developed as part of a major thrust initiated by the National Institutes of Health aimed at making medicine a more quantitative science [15]. Further, many more assays will undoubtedly follow in the near future as more genetic information is discovered by major research undertakings such as the Human Genome Project [16–19]. 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.

The paper begins with a basic discussion of the nature of genetic information followed by a summary of the most important procedures used for testing and reading this information. This is followed by some scaling arguments that favor the use of miniaturization. Next, a review of microscale protocols and their demonstration in microfabricated devices is presented followed by a discussion of important problems that must be solved for the implementation of practical engineering devices. The paper concludes with some projections on future research directions.

II. THE NATURE OF GENETIC INFORMATION

This section very briefly discusses the basic principles and terminology in molecular biology that are necessary for an engineering understanding of the task at hand. 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) [20]. 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 $4 \times 10^8$ 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(a). The sugar linkage gives the nucleotide directionality 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 com-

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plementary base pairs (G-C and A-T) forming a double strand (or duplex) as shown in Fig. 1(c). Double-stranded DNA is more stable in water because the hydrophobic bases are hidden by hydrophilic backbones.

All information relevant to cell growth and regulation is stored in this form. To perform an analysis, first the DNA must be extracted from the cell nucleus and purified. This procedure involves the disruption of cell membranes by chemical exposure to a detergent [21]. This is typically followed by purification using centrifugation or other methods for removal of cell debris leaving DNA in solution.

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 described below.

III. DNA Analysis Methods

Conventional genetic assay protocols use five fundamental chemical procedures for the analysis of DNA [22]. These procedures are the workhorses of molecular biology and must be reproduced in the microscale.

A. Chemical Amplification:

DNA samples are often present at concentration levels that are too low for any direct test. Therefore chemical amplification is used to increase the concentration of the sample. The amplification basically consists of a set of reactions that allow a DNA molecule or fragment to duplicate. Further amplification is hence obtained by repeating the procedure. Amplification factors as large as $2^{30}$ are common which permit detection based on single DNA molecules.

Several schemes can be used for amplification, but all of these use powerful enzymes. Enzymes are “miracle worker” protein catalyst molecules that can manipulate and modify DNA strands present in every living organism. In particular, polymerase enzymes assemble complementary strands of DNA from a single strand fragment. This enzyme scans single strands of DNA and, starting from a specific location, captures matching nucleotides from solution, and connects them to the single strand, assembling the complementary strand one base at a time like a zipper.

Molecular replication takes place when a double stranded fragment is first thermally separated (or denatured) into two strands followed by the polymerase action. This procedure is known as the polymerase chain reaction (or PCR) [23–27, 11] shown in Figure 2. A typical amplification uses the high-

![Fig. 1](image1)

![Fig. 2](image2)
displacement mechanism (SDA) [33, 34] can provide large amplification factors with the added benefit of isothermal operation. Conventional PCR amplifications are made in macroscopic thermal cycler accommodating multiple reaction vials. Macroscopic cyclers require roughly 90 minutes to complete the amplification; therefore faster thermal cyclers are desirable.

B. Fluorescence and Staining Visualization

The presence of DNA fragments is commonly detected by introducing in the mixture a suitable label molecule that binds to the fragment. Early labeling methods used radioactive $^{32}$P incorporated in the fragment nucleotides. Most modern labeling methods use fluorophore dyes that emit light when bound to DNA under external excitation [8, 35]. Light-emitting labels are extremely sensitive permitting the detection of individual molecules [36–38] in femtoliter samples. Hence these dyes are almost universally used for the visualization of DNA fragments. Both the type of bonding and excitation mechanism for these dyes varies.

Intercalating dyes such as Ethidium Bromide (EtBr) fluoresce when excited by UV light only when bound between two nucleotides in double stranded DNA as shown in Figure 3(a). Since a fragment can accommodate one intercalating label per base pair, a single DNA molecule can contain hundreds of fluorophores and emit a strong signal. Intercalating dyes affect somewhat the migration of fragments during separations; therefore for best accuracy sometimes a single fluorophore is attached at the end of the molecule.

Light emission can be excited in several ways. Conventional fluorophore labels require the excitation from UV light, hence the emission signal must be separated from the excitation using filters and dichroic mirrors. Light emission can also be excited chemically [39, 40] and by electrochemical reactions [41, 42]. Electrochemiluminescence (ECL) tagging methods use a large bright Ru(bpy)$_{3}^{2+}$/ end label that emits light in the presence of an electrochemical reaction. This technique is becoming increasingly important as it is regarded as the most sensitive [43, 44] tagging method.

DNA fragments with light-emitting tags can be observed using epi-fluorescence microscopes [45] with intensified PMT or cooled CCD cameras [46–48]. Chemiluminescent and electrochemiluminescent tags can be easily observed using conventional and cooled CCD’s [46, 49, 50].

C. Restriction Digestion

The analysis of replicated DNA often involves fragmentation (or digestion) of the molecule. Restriction endonuclease enzymes split double stranded DNA at specific locations. Restriction enzymes are essential for analyzing chromosome structure and sequencing very long DNA molecules.

Most enzymes recognize short DNA sequences of four to eight base pairs cleaving the fragment at this site at a point of symmetry. More than ninety enzymes have been characterized and are available for these operations [51, 52]. Restriction enzymes are used to cleave DNA molecules into specific fragments that are more readily analyzed. The size distribution of such fragments can serve as a fingerprint for a DNA molecule.

D. Electrophoretic Separations

Electrophoresis is a technique used for separating DNA fragments of different sizes from a mixture [53, 54]. DNA fragments in solution are negatively charged; therefore they drift under the presence of an applied field $E$ with velocity

$$v = \mu(N_{i})E$$

where $\mu$ is the fragment mobility. The mobility depends on the type of mobile phase and the fragment size $N_{i}$. Therefore if the mixture is introduced as a single band at a starting point in a mobile phase, the fragments are separated into bands composed of different sizes of DNA as they drift in a “race track” fashion as shown in Figure 4. The fragment separation is $\Delta L = \Delta \mu E t$,

![Fig. 3. Different type of DNA fluorescent dyes. (a) Intercalating dyes fit between two base pairs. (b) Single fluorophores can also bind to the end of the fragment](image1)

![Fig. 4. DNA fragments are separated into bands due to their different drift velocities. The calibration ladder serves as a reference.](image2)
gel. When the band width is determined by diffusional spreading with diffusion coefficient $D$, then [54]

$$N = \frac{\mu V}{2D}$$

(3)

Therefore high resolution separations require a high voltage $V$. This relationship is independent of the gel length but only holds for uniform fields. Typical macroscopic gels use voltages as high as 2 kV over distances of 20-100 cm. The maximum field is limited by both heating effects and the development of fragment co-migration. Conventional macroscopic gels are cast in the form of thin multi lane slabs. Due to Joule heating, electric fields in slabs are limited to 5-40 V/cm; hence good separations require many hours. Further, the slab gel preparation is labor intensive. Scaling of the slab is therefore greatly advantageous.

DNA fragments can also be separated in capillaries 10-300 μm in diameter [61]. This technique greatly benefits from the increased surface-to-volume ratio of the gel that permits the use of much higher fields and faster separations. The resolution of bands is further enhanced through the use of highly focused laser-induced fluorescence [62]. Typically, these capillaries are 50 cm long yielding high resolution separations in under one hour [61]. State of the art capillary electrophoresis (CE) systems use fields of up to 1200 V/cm achieving separation of 250 bands in 10 minutes [56, 63]. In CE systems, the capillary walls affect the sieving matrix polymerization and the separation quality; therefore for best results non-crosslinked gels are often used [60, 63–66, 55, 58].

E. The Sanger Sequencing Scheme

Replication techniques are combined with electrophoretic separations to devise a mechanism for reading the base pair sequence of a DNA fragment [67, 22]. Like PCR the duplication of the single stranded DNA starts at the primer location, but it is randomly terminated at locations where a specific base is present. The random termination is caused by the introduction of a small concentration of dideoxy nucleotides (ddNTPs) of one type (ddA, ddC, ddG, or ddT) in the initial mixture. These special nucleotides terminate the polymerase duplication when captured. The reaction thus generates complementary strand fragments terminated at all possible positions of the matching dideoxynucleotide.

In the Sanger scheme [67], four of these reactions are carried out with each termination base as shown in Figure 5. When these fragments are separated in a gel in four separate lanes, the relative positions of the fragments indicate the location in the sequence where a specific base is present; hence the sequence is read out directly. In this method, the sequence accuracy and the maximum readable fragment length depend on the gel uniformity and resolution. State of the art Sanger sequencing systems can read fragments about 1000 bases long using denaturing gels.

F. Hybridization Techniques

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 [68, 69, 8], 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 as 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 [70] as shown in Figure 6. The DNA probes can be either synthetic oligonucleotides or longer DNA fragments typically arranged in array form [71–73].

The application of these type of devices is wide and will probably extend further due to the massive parallelism present in large arrays. Several groups have developed theoretical methods for sequencing DNA on these devices [74, 75], but only sequencing of very short fragments has been achieved successfully [76]. Nevertheless the technique seems to hold some promise for longer fragments [77].

The primary difficulty associated with array hybridization techniques lies on the interpretation of the data. Most arrays have probes of short length (12-20-mer), but a typical fragment size distribution for a digested unknown does not necessarily match that for the probes. Therefore smaller fragments can hybridize to many probes, and the fluorescence pattern for differ-
ent fragment sequences is not unique. Also, short duplexes are stable under non-stringent conditions which favor the internal pairing of the sample [78]. Despite these pitfalls, the number of possible patterns can be very large; therefore excellent pattern matching can be achieved for finger-printing applications. To illustrate this point let a single strand of DNA be divided into \( m \) equal fragments each \( p \) bases long. Say for \( p = 8 \) and \( m = 20 \) under ideal hybridization conditions, in a complete, \( 4^p = 65,536 \) oligomer array, the number of distinct sequences that yield the same fluorescence pattern is

\[
N_s = m! \approx 24 \times 10^{18}
\]

(4)

and hardly unique (as required in sequencing). On the other hand the recognition error rate

\[
\epsilon = \frac{m!}{4^p \cdot m} \approx 10^{-78}
\]

(5)
is extremely low.

Hybridization arrays have been constructed on polypropylene [73], glass [72, 79], and silicon [80]. Reading of these arrays is typically done using confocal epi-fluorescence microscopes [81, 82] with cooled and intensified CCDs. Detection of hybrids is specially difficult as the size of the array pixels continues to shrink. Conventional CCDs have been used with radioactively labeled samples [83].

A typical DNA assay may involve a combination of the above techniques. For example the process may include the extraction of nucleic material from a cell followed by amplification, staining, restriction digestion, and electrophoretic separation or hybridization. The implementation of these protocols in the microscale hence requires microdevices capable of handling liquid samples, mixing, thermal cycling, and detection.

IV. BENEFITS OF MINIATURIZATION AND SCALING

In macroscopic protocols, relatively large microliter volumes of DNA are handled in vials and manually loaded into desktop thermal cycler 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 justification for the miniaturization of these systems in both clinical and research settings [84, 72, 85]. 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 7 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 the thermal cycle time while separation time Scales by \( S^{-1} \) (a much celebrated argument [86]). Ultimately, the minimum assay time is limited by the speed of the enzyme itself (30-100 bp/s [11]). 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 \( \mu \)m 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.

In recent years, several devices have been constructed that demonstrate the benefits of scaling. Some of these devices are rudimentary in construction, but they provide very convincing evidence that further miniaturization and systems integration should be pursued for this application.

V. MICROFABRICATED DEVICES

Over the past decade, the miniaturization of DNA assays has been investigated by several groups using a broad range of fabrication technologies and materials. While the construction for many of these devices is often rudimentary, these simple devices serve well as microscale protocol demonstrators.

A. Microfabricated Amplification Devices

A number of micromachined devices [87, 88] have been developed to accomplish faster 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 [87, 89] 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. 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 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 [90, 91]. 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 [93, 92]. These devices show a detection limit of 40 femtomoles of DNA, which is substantially less than that for commercial macroscopic devices.

**B. Electrophoresis Microdevices**

In 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 (≈ 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 [85, 94–99, 86, 100–106, 84, 107]. 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 [86]. 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 [108, 109].

Figure 11 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 and resulting in a high resolution separation. Typically the migrating bands are recorded using a confocal fluorescence microscope focused at a specific spot [95, 110] as shown in Figure 12. In the above scheme the injection plug length is enlarged due to lateral diffusion and convection eddies at the intersection [102, 111]. The cross channel voltage configuration can be changed to form a sharper plug. Figure 13 shows the injection plugs under two different schemes. In the top photograph, counter currents are injected into the separation channel to prevent the plug diffusion. The resulting plug volume is constant yielding higher resolution separations.

The performance of planar crossing channels for separations of DNA was demonstrated by Woolley [65]. Figure 14 shows separations of DNA fragments on a short, $8 \times 50 \mu m^2$, 3.5 cm long capillaries. About 450 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.

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 [61]. Unlike cross-linked polymers, these sieve 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 [112]. DNA separations are possible on silicon substrates because high voltage drops which may breakdown the isolation dielectrics [113] 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 15. The fabrication of these devices is discussed in section VI. On-chip detectors eliminate the need of expensive readout optics and open the road to low-cost disposable devices.

C. Micromachined Hybridization Arrays

Hybridization array devices can be micromachined using lithographic techniques [114, 80]. Fodor's technique [114] permits the photo induced solid synthesis of oligonucleotides as
shown in Figure 16. First, a glass substrate is coated with a linker molecule that has a photochemical removable protecting group [115, 116]. 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 [80]. The two layer process shown in Figure 17(b) is necessary to protect the oligonucleotide chemistry from the basic (OH− > 0.1 M) resist developer. The polyimide (XU-218, CIBA-Geigy) can be patterned after the resist development with a solvent.

The array probe patches range from 50-200 μm each; therefore a 1 cm² can contain 10-40,000 different probes. The number of possible oligonucleotides is much larger yielding, for example for a 15-mer probe, 415 ≈ 10⁹ combinations. Dense arrays with 20,000-96,600 probe patches have been reported [117, 118]. Finished arrays are mounted in flow-cell plastic packages. Figure 18 shows the example fluorescent array signature of a test for the HIV virus. These type of devices are now widely used in genetic research [82]. Currently, the fluorescent signal for these devices is read with a confocal fluorescence microscope as shown below

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 [119] fabricated a platinum electrode array device on a silicon substrate passivated with silicon nitride and a permeable agarose gel. Through the application of an electric field between the electrode and the sample, the hybridization time was reduced twenty five fold. The reduced time is attributed to the enhanced transport of charged molecules to selected micro locations, and the resulting localized increase in the sample concentration.

A seemingly useful property of field-directed oligonucleotide arrays is the capability for selective denaturing. This is important, as hybridization can still occur even in the presence of single base pair mismatches (SBPM) which makes the interpretation of the data difficult. By reversing the direction of the field and scrutinizing the field magnitude, it is possible to remove the incorrectly hybridized strand over perfect matching ones. Sosnowski [119] achieved electric field induced SBPM denaturing in a few seconds at a rate four times faster than denaturing of exact matches. In these devices, a multiplexed electrode plane is hence required; therefore high density electrically-addressable arrays will require a multiplexing circuit plane. A 128 microelectrode CMOS chip has been reported by Calliat [120]. In this device, the actual probe immobilization was directed to specific electrodes using an oligo-pyrrole scheme [121, 122].

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 [83]. A more practical implementation of these ideas is likely to be based on electrochemiluminescent labeling which lacks the adverse effects of radioactive sample handling.

### D. Fluid Processors

The devices above generally require sample preparation protocols involving dosing of reagents, mixing of reagents with the sample, and injection of the mixture into the device. These functions are performed by a fluid processor.

There is a substantial amount of literature on the miniaturization of these systems [123]. Most of these are made by complicated bonding techniques and many planes of functionality [124–131] for valves, gaskets, and pumping devices; therefore simpler and less expensive alternatives are desirable. Valves are a major source of difficulties in these systems. Micromachined mechanical valves are difficult to implement, require complex fabrication processes, and large operating power.

To date there have been only two implementations successfully applied to genetic assay systems. The first implementation uses a valve-less approach. In this system sample and reagents are transported in a liquid medium using electroosmotic flow controlled by external voltages. Therefore, the sample flow is controlled in much the same way as current in an electrical circuit. This technique was applied on glass chips for mixing, and labeling of DNA separation and labeling applications. This scheme is however not without pitfalls since PCR DNA amplification cannot be accomplished.

The second approach [132] uses pneumatic actuation for moving the sample and driving a set of passive valves. The device was micromachined on a polycarbonate plastic substrate. Plastic materials are beneficial for these devices because the diaphragm valves do not require special gasket materials.

The plastic processor consists of a set of chambers of different volumes interconnected by fluidic busses. Venting of excess gas is achieved using semi-porous hydrophobic membranes.

### VI. INTEGRATED TECHNOLOGIES

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 [133]. 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 [134]. Simple integrated devices have been fabricated by Woolley [133] and Burns [135]. 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-xylene. 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 [136], and the sample spread is controlled through a series of hydrophobic stops [137]. 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 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 [138–145]. These materials can be bonded or deposited on most substrates relatively easily [146]. Further, many plastic materials are bio compatible, 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 [138–140, 143, 144]. These microfluidic systems are partitioned into two stacked levels of functionality as shown in Fig. 23. 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-xylene [146], or combinations of p-xylene 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 24(a) shows a top photograph of plastic polyimide/p-xylene capillaries constructed on top of a regular CMOS circuit substrate. Figure 24(b) shows the cross section of p-xylene 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 °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 [139] fabricated CE channels with integrated detectors [135] as shown in Figure 25.

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 the Affymetrix processor [132], 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 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...
conventional CMOS circuits

Fig. 24. (a) Top view of polyimide/p-xylene channels constructed on top of a CMOS circuit. The low-temperature process does not degrade any of the circuit performance. (b) Example cross section of a p-xylene polymer channel [138–140].

Fig. 25. Capillary electrophoresis device fabricated using plastics on silicon. (a) The channel cross section is $200 \times 20 \mu m^2$. (b) Photograph showing separation of fluorescently labeled DNA fragments in plastic CE channel at elapsed times (HEC, 0.5%, 100 V/cm) [139].

present in these capillaries. Handique [137] used hydrophobic patches to stop the wetting of the solution, and a thermally expanding bubble to cut and propel individual sample drops. Man [140] 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 $100 \mu W$ of power to drive the sample forward.

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.

VII. PACKAGING, INTERFACING, AND STORAGE CHALLENGES

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. In this section we discuss areas of research that are important but have been neglected and propose several practical solutions.
Two important research areas that have not been addressed are chip packaging and interfacing, and reagent and chip storage. These important topics affect the operation of the device, and the cost of the testing apparatus and the assay.

A. Packaging and Chip Interface

These 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 mm³. 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 permits the injection of relatively large 5-10 μL samples with a syringe as shown in Figure 27. 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.

B. Chip and Reagent Storage

Unlike microelectronic devices, DNA assay devices use perishable reagents and enzymes. Therefore these devices can be operated in several modes that accommodate this constraint. In a possible scheme, the reagents are loaded after fabrication and preserved chemically or by physical means (dehydration, cooling, or freeze-drying). This scheme was reported by Albin [90] for PCR microwell arrays. Reagents, enzymes, and in-situ fluorescence reporters were dehydrated on each well after their introduction using an inkjet type device.

Alternatively, the reagents could be loaded just prior to the assay. Since the volume of the microfluidic part is very small, it seems that the first scheme may be difficult to achieve due to the gradual evaporation of the sample, especially when gel materials are involved. Larger reagent volumes could also be stored within a chip package, externally loaded by the testing apparatus. The latter option however is less likely since it would require relatively large volumes of reagents and enzymes. To date, practical ways to store these reagents have not been well developed. These are important topics of research as they affect the cost of the assay and reading equipment.

VIII. Summary

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. This paper provides a tutorial of the basic molecular biology protocols that must be reproduced at the microscale, reviews current microfabrication technologies and devices used for genetic diagnostics and discusses important technological and manufacturing challenges that must be addressed for the practical implementation of these devices.

REFERENCES


