Chapter 14
Biochip Platforms for DNA Diagnostics

Anil K. Deisingh, Adilah Guiseppi-Wilson and Anthony Guiseppi-Elie

Abstract This chapter looks at the use of microlithographically fabricated biochip platforms for DNA diagnostics and prognostics, although protein and RNA biochips are also briefly considered. Biodetection methods such as ion-selective electrodes (ISEs), microelectromechanical systems (MEMS) devices such as microcantilevers, optical, piezoelectric-based acoustic wave, and mass spectrometry are briefly discussed. Emphasis is given to label-free electrochemical (impedimetric, voltammetric, and amperometric) detection. The production of DNA biochips is highlighted as are the operation and design of the experiments to reveal gene expression and SNP data. Applications discussed include the monitoring of microbes, cancer classification studies, and patient stratification in drug development. Finally, challenges and issues facing the development of diagnostic and prognostic biochips are discussed in detail.

14.1 Introduction

Biochips, as the contraction implies, incorporate biologically derived recognition entities with the additive and subtractive techniques of microlithography in the production of useful analytical devices (1). Biochips are similar to but may be distinguished over biosensors by the density of functional recognition sites, generally $10^2 - 10^4$. Biochips are highly versatile devices that may be used for clinical
diagnostics and prognostics, patient stratification in drug development, disease management, and forensic applications, amongst others. These biochips automate repetitive laboratory tasks by replacing standard equipment with miniaturized microfluidic assays thereby providing very sensitive detection methods (2).

In recent times, three main types of biochips have become very important for diagnostic and prognostic purposes. These are nucleic acid (DNA, RNA, and PNA-based types) and protein biochips. DNA biochips may be classified according to the two types of nucleic acid probes affixed to the chip’s surface and by the density of such probes presented to a sample. Probes may be cDNA, generally derived from bacterial clone libraries, or oligonucleotides, prepared by solid-phase synthesis. Oligonucleotides may be in situ synthesized or presynthesized and then affixed to the chip substrate. One format for the DNA biochip uses a piece of glass, typically the size of a microscope slide, containing thousands of cDNA probes affixed to the glass platform within tiny (ca. 250 μm²) polyacrylamide gel pads (MAGIChip™, MicroArrays of Gel-Immobilized Compounds on a Chip (3)). When fully integrated with sample preparation, microfluidic sample management, and analyte detection, these systems are commonly termed ‘lab-on-a-chip’, which may allow for field-portable DNA analyses. DNA biochips are widely used for the detection of mutations in specific genes and to detect the differences in gene expression levels in cells (2).

RNA-based biochips were first reported by Breaker’s group at Yale (4). They used RNA-based molecular switches on a gold-coated silicon surface and arranged them in clusters. Each switch was able to bind to one specific target molecule and the researchers were able to identify different strains of E. coli. Protein biochips are being increasingly developed and several formats are available including (5):

(i) Antibodies arrayed to detect antigens from body fluids
(ii) Tissue extracts or purified antigens arrayed to detect serum antibodies or known proteins
(iii) ‘Bait’ proteins immobilized to detect interacting proteins
(iv) Large-scale proteome arraying of entire expression libraries encoded with fusion proteins

Generally, protein biochips are similar to DNA microarrays, being instead composed of arrays of immobilized proteins but with surface preparation and immobilization methods being far more complex and intricate in order to mitigate surface-mediated denaturation and to provide for preferential orientation of the active site or complement binding domain to the analyte in solution. In addition to DNA/RNA and proteins, subcellular organelles, cells, and tissues may also be the basis for biorecognition in biochips.

This chapter, however, concentrates on biochip platforms for DNA diagnostics. Considered topics include biorecognition moieties, biodetection methods, microarrays, opportunities for biochip diagnostics and prognostics, and their uses in patient stratification and drug development. The chapter concludes with a discussion of the key issues related to the production and use of diagnostic and prognostic biochips.
14.2 Molecular Biorecognition

Several biomolecules can be used as recognition moieties in the development of DNA biochips. These include cDNA, oligomeric DNA, and PNA as well as RNA. Integrated with solid-state devices, these biomolecular recognition entities need to be immobilized, stabilized, and presented to targets so as to maximize hybridization kinetics and hybridization fidelity as well as maximize the subjacent device sensitivity.

There are five main classes of general immobilization techniques:

(a) Retention by an inert membrane.
(b) Physical adsorption at a solid surface.
(c) Cross-linking with bifunctional agents such as glutaraldehyde, hexamethylene diisocyanate, and others.
(d) Physical entrapment within polymer matrices such as polyacrylamide and cellulosics.
(e) Covalent coupling to a functionalized solid-state support such as polystyrene, silicon, glass, or metal: this method is the most irreversible of the techniques.
(f) Covalent coupling to and entrapment within polymer matrices such as within hydrogels.

The major advantages of irreversible immobilization are:

(a) It may allow for reuse of the device or system.
(b) The immobilized molecule may be more stable than the solution species.

Regardless of the approach taken, the goal is to harness the specific recognition properties of biomolecules. This can be done either discretely or as part of a more complex system so that the recognition reaction is linked to a physicochemical transduction device and made functionally integral to an analytical system.

14.3 Biochip Transduction Methods

The wide range of possible detection methods gives the biochip its diversity. These include electronic devices (such as field effect transistors); microelectromechanical (MEMS) devices such as micro and nanocantilevers; simple metallic and semiconductor electrodes for electrochemical (amperometric, voltammetric, and impedimetric) detection; optical devices including fibers and fiber bundles (for absorption, fluorescence, luminescence, and chemiluminescence); quartz-crystal oscillating devices; and mass spectrometry. Amongst these methods are those described as direct and others that are indirect. Direct methods derive their signals from the hybridization reaction directly, for example, quartz crystal oscillators, impedimetric biochips, micro- and nanocantilevers, or interferometers. These detection methods exemplify responses that require no label and are therefore also referred
to as label-free methods. Although label-free, their signals may be enhanced, limits of detection lowered, sensitivity increased, and dynamic ranges attenuated by the use of nanoparticle or chemically responsive labels. Indirect methods derive their signals from the measurement of a label, for example, a radioactive isotope or a fluorescence label. Each of these methods is briefly described in the succeeding paragraphs.

Ion-selective electrodes (ISEs) have allowed for greater specificities to be achieved, especially for single-use applications. In the 1970s, improvements in semiconductor technology led to the development of the field-effect transistor (FET) that usurped the ISE platform. The FET is a very high impedance transistor and most sensitive measurements of small potentials requiring very low currents are made using this technology. However, the FET proved expensive to produce and as such has yet to achieve its full commercial potential; in no small part because of difficulties with regard to its reliability in operation. Development and commercialization of pH-FETs and the emergence of organic thin film field effect transistors (TFTs) suggests that DNA FETs may become a technological and commercial possibility.

MEMS devices are proving popular as a modern method for the detection of biomolecules. They may combine mechanical parts, several unit operations, sensors, actuators, and electronics on a common silicon substrate through the utilization of microfabrication technology (6). This technology allows the integration of silicon-based microelectronics with micromachining approaches and sophisticated detection systems to allow for the development of complete systems-on-a-chip (6). Some of the enabling technologies allowed by MEMS include polymerase chain reaction (PCR), microsystems for DNA amplification and identification, biochips for the detection of chemical and biological agents, and microsystems for high-throughput drug screening and selection (6).

Electrochemical methods are generally very accurate and sensitive instrumental techniques. One of the most widely used methods is voltammetry, which makes use of a microelectrode such as a platinum wire. The potential of the electrode is varied, generally in a linear sweep, and the resulting current is recorded as a function of the applied potential. The microelectrode restricts the current to a few nanoamperes with the result that the concentration of the test substance in solution remains essentially unchanged after the experiment (7). Amperometry is the application of voltammetric measurements at a fixed potential to detect changes in the current as a function of the chemical potential of an electroactive species. Amperometric electrodes are made on a microscale (≤50 μm diameter) which allows for enhanced mass transport independent of flow, an increased signal-to-noise ratio, and measurements in high-resistance media (8). As described later, electrochemical methods allow for miniaturization of instruments for use in DNA hybridization (9).

One of the reasons for the rapid progress of the biochip field is the advances in the optics arena. The evanescent wave phenomenon is one such example. When light is reflected at an optical interface where there is a change in the refractive index, there is a decay of energy away from the point of reflection into the surrounding
medium (10). When the evanescent wave is used to excite fluorescent molecules bound to the surface of the waveguide, the phenomenon is termed total internal reflection fluorescence (TIRF). If, however, there is an excitation of the electron plasma of a thin metal layer covering the surface of the waveguide, the process is called surface plasmon resonance (SPR). Both these phenomena, because of their considerable surface sensitivity, have received considerable attention in the development of DNA biochips.

Another major development has been the introduction of optical fibers and optical fiber bundles. Optical fibers have an outer, black polymeric sheath that provides protection, avoids coupling of ambient light through the walls, and gives mechanical support and strain relief to the inner core. In fiber optics, the detection circuitry is completely electrically isolated from the point of measurement, which has important safety implications for clinical diagnostic devices. In fiber optic devices, it is possible to use small polymer microspheres coated with an absorbing or fluorescent dye. These spheres can be placed within a hollow tube fixed onto the end of the fiber bundle or attached directly to the surface of the fiber optic (11,12). By using these microspheres, there is increased surface area available for immobilization of biorecognition molecules and interaction with the sample.

The application of piezoelectric-based acoustic wave devices to DNA detection has also been an important development. These are sensitive to changes in mass, density, viscosity, and acoustic coupling phenomena and, therefore, series resonance frequency can be used as a sensitive transduction parameter (13). These piezoelectric crystal devices generate and transmit acoustic waves that are dependent on frequency. Quartz is the most frequently used piezoelectric crystal because it can act as a mass-to-frequency transducer. AT-cut crystals (+35° 15′ orientation of the plate with respect to the crystal plane) are favored because of the excellent temperature coefficients in the range 10–50°C. One of the first sensors to be introduced from piezoelectric materials was the thickness-shear mode (TSM) sensor, which, if the substrate is quartz, may commonly be termed the quartz crystal microbalance (QCM) or bulk acoustic wave (BAW) sensor.

Mass spectrometric methods are becoming very important with these including standard approaches such as electrospray ionization and matrix-assisted laser desorption ionization (MALDI). Recently, newer techniques have started to make an impact and these include:

(a) Ion mobility spectrometry (IMS) which has the ability to separate ionic species at atmospheric pressure. Research is currently underway to develop low-pressure IMS systems.
(b) Atomic pressure ionization (API) and proton transfer reaction (PTR) techniques. Both are rapid, sensitive, and specific and allow measurements in real-time. In addition, they do not suffer drift or calibration problems.

A key feature of each detection modality is its ability to produce a measurable response, whether directly or indirectly, that is above noise and is consequent to the DNA hybridization reaction.
14.4 DNA Microarrays

Microarray technology “promises not only to dramatically speed up the experimental work of molecular biologists but also to make possible a whole new experimental approach in molecular biology” (14). DNA microarrays exploit an ordered, two-dimensional presentation of biorecognition probe entities, fluorescence tagging of targets, and scanning confocal imaging of the recognition–target complex or hybridization product. The most attractive features of microarrays are throughput and consequently, speed of analysis. The potential for miniaturization with its attendant reduction in sample volume does exist, but is still to be fully realized. This allows DNA microchip technology to have great potential for rapid multiplex analysis of nucleic acid samples. Examples of these include the diagnosis of genetic diseases, detection of infectious agents, measurements of differential gene expression, patient stratification in drug development, drug screening, and forensic analysis (15).

These chips are often fabricated from glass, silicon, or plastic supports and are usually composed of thousands of reaction zones (10–250 μm in diameter) onto which individual cDNA and/or oligonucleotides have been deposited and tethered. This results in densities up to $10^6$ sites/cm$^2$ in a typical 1–2 cm$^2$ chip (16). These high-density DNA arrays will usually require the use of physical delivery such as microjet deposition technology, which involves the dispensing of picoliter volumes onto discrete locations on the chip (15). In addition, contact pin arraying (spotting of 75–150 μm diameter spots) and microsolenoid dispensing (dispensing of 150–250 μm diameter spots) are also widely used. However, high-density DNA arrays generally benefit from the activation of the surface for a covalent attachment of the cDNA or oligonucleotide probes.

The successful development of DNA chip technology requires a multidisciplinary approach with various technical requirements to be satisfied. These include the development of algorithms and informatics for defining oligonucleotide probe sequences and lengths, methods for fabricating the probe arrays, detecting the target hybridization, algorithms for analyzing the data, and reconstructing the target sequence (15). As may be discerned, these are not simple procedures and careful experimental work is a necessity. With the advent of automated gene chip systems, there has been a decrease in the time required for analysis and there has been a reduction of human error.

In general terms, a microarray for gene expression analysis works by exploiting the ability of a given mRNA molecule to be reverse transcribed and have the resulting cDNA RT product successfully hybridized with high fidelity to the complementary strand of the DNA template from which it originated. If the microarray contains many DNA probes (typically 1–3 $\times$ 10$^4$), it is possible to determine the expression levels of thousands of genes within a cell, performed in a single experiment, by measuring the amount of cDNA bound to each site on the array (17).

To achieve meaningful results with microarrays, it is necessary to invest in proper design of the experiment. Usually, the following steps are involved (17).
(i) Prepare the DNA chip using the probe DNA molecules (cDNA or oligonucleotides).
(ii) Isolate the mRNA from the properly defined cells or tissue and evaluate this for extent of degradation if any.
(iii) Perform a reverse transcription (RT) and fluorescence labeling of the RT cDNA product. This may be achieved via in situ labeling using d-NTPs or posttranscriptional labeling using amino allyls.
(iv) Generate a suitable hybridization buffer solution containing a mixture of the fluorescently labeled cDNAs.
(v) Incubate the hybridization mixture containing the fluorescently labeled cDNAs with the DNA chip.
(vi) Using confocal scanning laser technology, the bound cDNA is detected and the acquired data stored on a computer.
(vii) Analyze the data using informatics tools and approaches established by the design.

The fluorescent tags are excited by the laser and, by using a confocal microscope and camera in tandem, a digital image of the array is created.

To date, microarray technology, a largely semiquantitative analytical technique, has been most valued in the basic research arena (18). Studies using microarrays have served to advance understanding of disease processes, and as the technology evolves it will become a tool for clinical medicine, providing a rich source of information on disease susceptibility, diagnosis, and prognosis. As a research tool, DNA microarrays have already been used in the study of heart, blood vessel, and lung disease; cystic fibrosis; human immunodeficiency virus (19); cancer (20); and single nucleotide polymorphisms (21). They have been used more broadly as well, to study arabidopsis, rat, yeast, and E. coli genomes and mouse models. Additionally, DNA microarrays are being increasingly used to monitor gene expression in humans (15). Researchers have used RNA expression in biochips to identify differential gene expression relevant to different biological states. Clontech (Palo Alto, CA) have produced the Atlas™ microarrays that provide sensitive detection of gene expression by using fluorescent dyes and glass or nylon substrates (15).

The evolution of microarrays is to use the information gleaned from genomic microarrays in the development of pathway-specific, or diagnostic/prognostic microarrays that employ smaller suites of genes in highly focused assessments (22). This evolution towards so-called “theme arrays” has begun. SuperArray Bioscience Corporation has developed the Human Th1-Th2-Th3 Gene Array which contains 96 genes relevant to understanding helper T cell biology. These genes include the cytokines specifically expressed by both Th1 and Th2 subtypes. The array also contains genes encoding transcriptional factors that regulate the expression of these cytokines as well as other markers of CD4+ T lymphocytes. Simple side-by-side hybridization allows relative expression of these genes in experimental RNA. Related products include: Cancer Drug Resistant and Metabolic; Common Cytokine; Inflammatory Cytokines and Receptors; Chemokines and Receptors; Interleukins and Receptors. IntelliGene™ DNA microarrays are medium-density
cDNAs arrayed on standard 2.5 cm × 7.5 cm glass slides for standard dual color analysis using high-resolution fluorescent detection. Arrays target human cancer, human cytokines, endocrine disruption, as well as cyanobacterial ORF, *Arabidopsis*, and mouse and *E. coli* gene analysis. This movement towards confocally imaged, targeted microarrays is converging with DNA biochips that use detection technologies other than confocal fluorescence imaging.

One of the areas where the use of DNA microarrays is proving to be of immense importance is in the monitoring of microbes. These can serve as detection and identification tools for clinical applications. Commercially available biochips include (23): *Helicobacter pylori* arrays from MWG Biotech; *Mycobacterium tuberculosis*, *Plasmodium falciparum*, and *Candida albicans* chips from OPERON; *E. coli* arrays from Pan Vera; *E. coli, Pseudomonas aeruginosa* and severe acute respiratory syndrome (SARS) biochips from Affymetrix; and *E. coli* and *M. tuberculosis* arrays from Sigma-Genosys. Other companies such as Siebersdorf Research (Germany) and Agilent will create custom-made chips for the organism of interest (23). In a related area, Nanogen Inc. (San Diego, CA) has developed an electronically controlled sample preparation process for the dielectrophoretic separation of *E. coli* from blood cells. The bacteria are then lysed by high-voltage pulses. The company has fabricated several microelectronic chips including those containing 25, 100, 400, 1600, and 10,000 addressable test sites. The latter is being developed for drug discovery methodologies.

In a major academic development, researchers at St. George’s Hospital Medical School in London have formed a group called BμG@S (Bacterial Microarray Group at St. George’s). This has been funded by the Wellcome Trust and brings bacterial genome researchers around the world into an organization to develop whole-genome arrays for 12 pathogens including *Campylobacter jejuni, Haemophilus influenzae, Yersinia pestis,* and *Salmonella typhi* (23). This collaborative effort is expected to have immense benefits as the collective research will generate more results on a faster basis. These can then be used to lead to more efficient identification procedures that will have a positive impact on health care systems.

Bekal and co-workers (24) have described a method for the rapid identification of *E. coli* pathotypes by virulence gene detection. An *E. coli* virulence factor DNA microarray composed of 105 DNA PCR amplicons printed on glass slides and arranged in eight subarrays corresponding to different *E. coli* pathotypes was developed. Fluorescently labeled genomic DNA sequences were hybridized to the virulence gene microarrays for optimization and validation. It was reported that this type of microarray is a powerful tool for both gene quantitation and subtyping.

Finally, *Listeria* species have also been identified by a microarray-based assay. Six species of *Listeria* were amplified by a multiplex PCR and subsequently hybridized to individual oligonucleotide probes specific for each *Listeria* species (25). It was demonstrated that the method allowed unambiguous detection of all six species based on sequence differences in the iap gene. The authors concluded that microarrays are valuable for the identification and characterization of bacterial pathogens. It must be borne in mind, however, that the huge amount of data generated from these microarrays will require experts to interpret the results.
DNA microarrays have also been used for the identification of viruses, although this area is still in its infancy. Recent applications include the detection of the human immunodeficiency virus (HIV) responsible for AIDS (26) and the influenza virus (27). In the former, two methods of nucleotide sequencing were compared for the detection of drug resistance mutations in HIV type 1 reverse transcriptase (RT) in viruses isolated from highly RT inhibitor-experienced individuals (26). It was found that of the 11,677 amino acids deduced from population PCR products by both cycle sequencing and sequencing by hybridization to high-density arrays of oligonucleotide probes, 97.4% were concordant by both methods with discordances mainly due to genetic mixtures within or adjacent to discordant codons. For isolates evaluated by additional sequencing of molecular clones of PCR products by both methods, the discordance between methods was less frequent.

In the latter example, a model DNA microarray was shown to facilitate typing and subtyping of human influenza A and B viruses (27). RT-PCR was used to prepare cDNAs encoding about 500 bp influenza virus gene fragments that were cloned, sequenced, reamplified, and spotted onto a glass support. The target DNAs included multiple fragments of the hemagglutinin, neuraminidase, and matrix protein genes. Cy3- or Cy5-labeled fluorescent probes were hybridized to the target DNAs and the arrays were scanned to locate the probe binding site(s). The researchers indicated that the hybridization pattern agreed well with the known grid location of each target and the signal-to-background ratio varied from 5 to 30. No cross-hybridization could be detected. With further testing, this method may prove to be clinically useful. Further differentiation of amplified molecules in complex mixtures can be obtained by hybridizing combinations of Cy3- and Cy5-labeled DNAs. A particularly attractive feature of this research is the identification of several sets of multiplex primers that collectively target influenza A and B virus strains. This allows DNA microarray technology to act as a supplement to the information obtained from PCR-based diagnostic methods.

Additionally, researchers at the Center for Biologics Evaluation and Research and at the National Institute of Allergy and Infectious Diseases have developed a system to identify isolates of rotaviruses. These organisms are responsible for causing diarrhea in infants in the developing world (28). Five clinically relevant G genotypes (G1 to G4 and G9) were studied. The genotype-specific oligonucleotides on the glass slides were allowed to bind to multiple target regions within the VP 7 gene which are highly conserved among individual rotavirus genotypes. Nested PCR was performed and the identification of rotavirus genotype was based on hybridization with several individual genotype-specific oligonucleotides. An advantage of this microarray approach over PCR is that there is unambiguous identification of all rotavirus serotypes (28). This approach combines the high sensitivity of PCR with the selectivity of DNA–DNA hybridizations. Furthermore, the presence of random mutations allows each individual virus isolate to produce a unique hybridization pattern. This allows the differentiation of different isolates of the same genotype.

De Risi’s lab (29) has reported on an approach for highly parallel viral screening. A long (70 mer) oligonucleotide DNA microarray capable of simultaneously detecting hundreds of viruses was developed. The different viruses were detected
by using virally infected cell cultures and related viral serotypes were distinguished by the unique hybridization pattern of each virus. This offers an advantage over existing techniques for screening a broad spectrum of viruses as it allows for the comprehensive and unbiased analysis of viral prevalence in a biological setting. In addition, individual viruses which were not explicitly represented on the microarray were detected indicating that this method may find use in virus discovery. An important aspect of this research was the ability to detect multiple viruses in human respiratory specimens without the use of sequence-specific or degenerate primers. This method allows for discrimination among viral subtypes.

DNA arrays have also found use in cancer classification studies. With over 200 different types of cancer, it is difficult to differentiate some cancer subtypes; for example, cells of acute myeloid leukaemia (AML) and acute lymphoblastoid leukaemia (ALL) look very similar. It is essential to achieve a correct diagnosis as the treatment regimens may be different. It has been reported that, by analyzing the expression patterns of 50 genes on an array representing about 7000 genes, accurate classification was possible for both AML and ALL. Also, the array allows a more robust diagnosis than tests based on a single protein or the activity of one enzyme. In another application, DNA microarrays were used in the discovery of new cancer subtypes; for example, analysis revealed two previously unrecognized subtypes of non-Hodgkins lymphoma.

Gene expression patterns in renal cell carcinoma were assessed by complementary DNA microarray (30). Renal cell carcinoma comprises several histological types each with different clinical behavior and thus accurate pathological characterization is essential. The authors describe gene expression profiles in 41 renal tumors determined by DNA microarrays containing 22,648 unique cDNAs representing 17,083 different UniGene Clusters, including 7230 characterized human genes. Differences in the patterns of gene expression among the different tumor types were readily observed (30).

The genomic classification of brain tumors and brain tumor subtypes is also being pursued at the Center for Bioelectronics, Biosensors and Biochips and the Neuroanatomy Project. Using a 10,000 gene spotted oligonucleotides (50 mers) microarray, the expression profiles of various tumor types are being defined. Such an effort involves very close working among bioengineers, pathologists, biostatisticians, and neurosurgeons. As a result of the semiquantitative nature of today’s microarrays, the likely diagnostic arrays of the future must be brought into a more quantitative and clinically relevant format (31). Also, the diagnostic community will need to accommodate decisions and interventions based on quantitative risk-based assessments in a fashion similar to that of the environmental community.

To achieve these goals, the design and fabrication of the microarrays themselves need to be brought into a more quantitative framework with greater attention paid to well-defined and highly reproducible surface chemistries, DNA probe attachment protocols and procedures, and more rigorously defined hybridization protocols (32). Furthermore, present biochip readers are slow and expensive. Current confocal scanning methods address signal quality issues optically. However, electrical signal processing methods can also be used to reduce interference. These include cross-talk
cancellation and multiplexing schemes. Plextek (Essex, UK) claims that further improvement can be obtained by the design of a linearized detector sensitivity control. The current emphasis on statistical manipulation of data to address engineering limitations and interoperator variability that contributes noise, poor precision, and poor accuracy needs to be addressed by improved design, fabrication, and implementation protocols of DNA microarrays.

Current normalization techniques implemented in most software for microarrays assume that fluorescent background within spots is essentially the same as that found throughout the microarray slide and can be measured by fluorescence surrounding the spots (33). This assumption, however, is not valid if the background fluorescence is spot-localized and inaccurate estimates of background fluorescence under the spot create a source of error, especially for genes of low transcript copies (33). Such nonspecific adsorption of target cDNA to oligo or cDNA probe spots may be addressed by including Arabidopsis genes on human microarrays and subtracting the intensity of the nonspecifically adsorbed signal from the probe signal. Finally, DNA detection schemes using metal nanoparticles or quantum dots as labels, rather than fluorescent organic dyes that are subject to photobleaching, have been developed. These are based on the physical properties of metal nanoparticles: large extinction and scattering coefficients, catalytic ability, surface electronics, and efficient Brownian motion in solution. These properties have resulted in nanoparticles overcoming some of the limitations of fluorescent labels such as cost, ease of use, selectivity, and sensitivity.

14.5 Opportunities for DNA Biochips

Opportunities for applications of DNA biochips are most attractive in the area of human health and specifically in the area of personalized medicine. The R&D investments, marketing, and sales and distribution costs, given today’s models for development of technologically driven companies, do not support similar opportunities in the environmental or industrial biotechnology sectors. The human health sector presents opportunities for near-patient (bedside), physician’s office, clinical laboratory, and molecular diagnostics laboratory (hospital). In the short term, diagnostic biochips will likely have more impact in the area of drug development than in clinical molecular diagnostics. However, clinical molecular diagnostics represents a looming opportunity separated only by the regulatory challenges and broader societal concerns.

14.5.1 Diagnostic Biochips and Links to Drug Development

A primary goal of drug research is to determine, on as small a sample as possible, and for as many different molecule pairs as possible, the formation of a biologically
active complex. Pharmaceutical companies must perform biochemical assays during all phases of the drug development cycle; from the initial screening of libraries of available compounds, through the focused evaluation of promising new drug candidates, to the clinical testing of drugs being readied for market. These assays are poised to become more important as the industry moves towards the delivery of drugs that target specific genetic profiles. A critical element in this process is the comprehensive evaluation of patient response based on molecular indicators. It is unlikely that populations will be prospectively screened, stratified, and selected prior to joining a study, which limits market size and may even be socially objectionable. It is more likely that subpopulations identified by the study will be screened retrospectively to establish a logical genetic basis for exclusion of nonresponders and identification of overresponders within the target population (34). This limits the cost associated with development and is less disruptive of the well-established drug development process. A key feature will be the procurement and banking of tissue and/or fluid specimens from patient participants in the study. Diagnostic biochips will feature prominently as a means for providing the critical molecular data for drug discovery (21), and improving the drug discovery process by enabling proper patient stratification during clinical trials.

One of the most important developments in recent times has been the use of single nucleotide polymorphisms (SNPs pronounced “snips”). A SNP is the most common type of variation in the human genome and it arises as a result of a single base difference in the coded protein between individuals. These variations occur about once every 1000 base pairs in the genome, making up the majority of the three million variations in the genome. Furthermore, the frequency of a particular polymorphism remains stable in the population. Usually, the effect of a single SNP on a gene is not large but small effects can influence susceptibility to common diseases such as Alzheimer’s and the risk of heart disease. Oligonucleotide (DNA) chips can be used to discriminate between alternative bases at the site of a SNP. These DNA chips allow many SNPs to be arranged in parallel, which is necessary for large-scale association or pharmacogenomic studies. A DNA sequence containing a SNP is hybridized to a DNA chip and discrimination of alternative bases (termed “typing”) is carried out at the polymorphic site. The signal that corresponds to the specific identified base is detected.

Two typing methods are widely used. The first uses allele-specific hybridization where short DNA sequences on the chip represent all possible variations at a polymorphic site. A labeled DNA will only stick if there is an exact match. The fluorescent signal indicates where the base is located. Secondly, the oligonucleotide on the chip may stop one base before the variable site and typing depends on allele-specific primer extension. A DNA sample attached to the chip is used as a template for DNA synthesis with the primer being the immobilized nucleotide. The four nucleotides, to which are added fluorescent labels and DNA polymerase, are also introduced. The incorporated base is identified by the fluorescent signal, although mass spectrometry may also be used. These methods are suitable for high-throughput SNP typing, usually for large-scale studies of populations. Two applications are: (a) association studies, which try to correlate SNP profiles
with susceptibility to disease, and (b) pharmacogenomic studies, which link SNP profiles with drug response patterns. A disadvantage of these chip-based assays is that new SNPs cannot be readily incorporated onto the chip. However, by using bead arrays, this problem is being solved.

In 1999, a SNP consortium was set up by the Wellcome Trust in collaboration with ten big pharmaceutical companies and several genome research institutions. The goal was to produce a public database of SNPs that could be easily accessed in the fight against major disease. Each company contributed US $10 million to this endeavor and by September 2001, 1.5 million SNPs (five times the original anticipated number) were discovered (35).

Recently, a new initiative has been established to provide a haplotype map of the genome. Within the human genome, different genetic variants within a chromosomal region (haplotypes) are not found in all possible combinations; certain combinations are more common than others. Differences in haplotypes may be associated with different susceptibilities to disease and, by mapping the haplotype structure of the genome, the genetic basis of some diseases may be identified. A five-nation consortium of Canada, China, Japan, the United Kingdom, and the United States, has pledged US $100 million over three years (2002–2005) to construct a haplotype map based on 200–400 genetic samples from each of four different populations: the Han Chinese; the Yorubas in Nigeria; the Japanese; and individuals in the United States with northern or western European ancestry. It is hoped that the haplotype map will simplify the search for medically important DNA sequence variations and provide insights into human population structure and history.

Haplotypes can be used for the mapping of disease genes. This is possible because mutations responsible for a genetic disease always enter the population within an existing haplotype (“ancestral haplotype”). Over several generations, recombination events occur within the haplotype but the disease allele and the closest SNPs will still be inherited as a group. Once this haplotype can be identified in a group of patients with the disease, typing the alleles within the haplotype allows a conserved region to be identified. Because many SNPs are present, genes can be mapped accurately. The International HapMap project aims to speed the discovery of genes related to common illnesses such as asthma, cancer, diabetes, and heart disease (36).

An alternative to genotyping is phenotyping. Thus, an antidepressant drug such as fluoxetine (Prozac®) is metabolized by the cytochrome P450 family of enzymes to produce nor-fluoxetine. Both drug and metabolite molecules will appear in the urine. Using a recombinant form of the enzyme in a suitably designed biochip allows the analysis of two orders of magnitude in variation of metabolic activity found in the population for this drug. Companies such as ABTECH Scientific are currently researching this approach, and although it does not measure DNA directly, it gives an indication of the clinical manifestation of this variation in the population. The core technology is based on interdigitated microelectrodes, which are arrays of tiny electrodes derived from metal sputtered onto a substrate (37). A microlithographically fabricated chip that can detect biological relevant analytes has already been produced.
14.5.2 Diagnostic Biochips for Clinical Molecular Diagnostics

The era of personalized molecular medicine is here (38). Ushered in by a now universal appreciation of the molecular basis of disease, molecular analyses, both genetic or proteomic, to improve a patient’s outcome in the management of his disease, is revolutionizing healthcare. With the potential to (i) optimize therapy and enable appropriate patient dosing, (ii) detect diseases when in their early stages, (iii) greatly reduce adverse drug reactions and drug–drug interactions, and (iv) potentially improve patient compliance, clinical molecular diagnostics is a reality of growing significance (39).

Several clinical molecular diagnostic (MDx) areas are rapidly emerging; (i) MDx for the optimal drug dosing of patients, that is, the selection of the patient-specific dosage for a more universal therapy; (ii) MDx for the selection of patient-specific therapy, that is, the application of therapeutic drugs to patients who by the presence or absence of a particular biomarker will have a high likelihood of favorable drug response; and (iii) MDx for the molecular grading and staging of disease, particularly cancer, that is, the development of diagnostic and prognostic tests to more accurately predict patient outcomes to multistage disorders. In many cases, differential drug response arises from subtle differences in the genes that code for the production of drug-metabolizing enzymes, drug transporters, or molecular drug targets such as receptors. Particularly noteworthy amongst drugs demonstrating differential drug response are those drugs metabolized by the liver enzyme, Cytochrome p450. This is because of the very large variation in CYP activity that is apparent in the population.

Related in principle to patient stratification in drug development, the development of diagnostic tests to measure specific protein levels or gene copy numbers has allowed the emergence of such drugs as Herceptin® and Gleevec®. Herceptin is an antibody drug that specifically inhibits the cell surface protein, human epidermal growth factor receptor 2 (HER2). This receptor has been found to be overexpressed in approximately 30% of breast cancer patients histopathologically defined classes. Molecular diagnostic tests now exist that can measure HER2 protein levels or gene copy numbers and so allow an identification of that subset of patients for whom Herceptin is indicated. The beneficial corollary is that women who are HER2-negative need not be given Herceptin as a first course of treatment.

Gleevec is a tyrosine kinase inhibitor that specifically binds to the ATP binding site and inhibits the action of the abnormally formed BCR–ABL protein, a kinase enzyme. The BCR–ABL produced kinase is responsible for an uncontrolled increase in white blood cell population; the basis for chronic myelogenous leukemia (CML) (40). The BCR–ABL protein is itself a fusion of two normal proteins that results because of a chromosomal rearrangement. A molecular diagnostic test for the gene that codes for BCR–ABL allows targeted prescription resulting in improved response rates, lower toxicity, and a high probability of complete remission (41). There are many other highly regarded examples, some already available and several yet emerging, that foretell the success of molecular medicine and personalized care. Such examples are most persuasive when the diagnostic test is
linked to an available therapy. When such MDx tests are not accompanied by an established therapy they may be the source of frustration for patients, providers, and insurers alike. However, such tests, during a period of ambivalence may well be the basis for proper patient stratification that enables the future development of appropriate therapies.

Another source of complication arises for those diseases and disease states that are multigenetic. Here the challenge is first arriving at a suitable panel of genes that may be diagnostic and prognostic for the disease and its outcomes. Second, is the challenge of implementing that panel of genes along with suitable analytical controls onto a suitable geneosensor or biochip platform. The first challenge may be defined in part as the challenge of class comparison, that is, the identification of genes that are differentially expressed among the predefined classes of that disease. The first challenge may also be defined in part as the challenge of class prediction, that is, the identification of genes that are differentially expressed among patients for whom the disease outcomes are or may become known. In both cases the technical challenge is exacerbated by the likely emergence of genetic subclasses revealed by the very attempt at class comparison and by the multiple interventions that affect patient outcomes. A looming question then is whether the health care community is prepared to accept a risk based on a deterministic paradigm for health care in an era of molecular diagnostics and personalized medicine.

14.5.3 Biochip for Diagnostic Classification and Prognostic Stratification of Primary Brain Tumors

One important nexus for future DNA microarrays is found in clinical diagnostics and prognostics. This entails the development of diagnostic tools based on microarray technology that utilize a targeted suite of genes directed at specific diagnostic screening applications (42). At the Center for Bioelectronics, Biosensor and Biochips we are engaged in the development and deployment of a diagnostic and prognostic biochip for primary brain tumors. This biochip seeks to combine a focused panel of genes that is capable of genetically delineating the several World Health Organization (WHO) histopathological classes of primary brain tumors (astrocytomas) with microfluidics technologies that aims to support the several unit operations needed to realize a “tissue-in-data-out” bioanalytical paradigm. Two parallel efforts were established: the conduct of retrospective genetic class comparison, class delineation, and class prediction studies using differential gene expression profiling of tissues taken from the Tissue Data Bank at Virginia Commonwealth University; and the development of electroanalytical techniques that may be suited for rapid hybridization detection of transcripts in a clinical molecular diagnostics format (43).

Microarrays are now being widely applied to the study of differential gene expression profiles to improve class prediction for many different cancer types, including colon, lung, esophageal, and breast cancers. Various statistical methods have been
developed to allow improved class prediction using microarray data. In parallel, there is considerable attention now being given to rendering microarray data quantitatively more rigorous by (i) optimization of oligonucleotide probe length and definition relative to the 3′ end, (ii) reproducible covalent immobilization of probes to qualified surfaces resulting in exacting surface coverage, (iii) minimizing nonspecific adsorption of targets to reference areas, (iv) the implementation of a larger number of control features, and (v) the development of advanced reagent sets that allow improved detection of low copy number transcripts and more stringent hybridizations.

The C3B 10k oligonucleotide microarray was designed using the MWG 10kA human oligonucleotide library (Cat # 2190-000000, MWG) as the base gene library. Seventeen additional “housekeeping” genes and eleven control genes that are found on the Affymetrix Hu133A chip (Table 14.1) were added to the 9984 5′-C₆-amine-terminated and HPLC purified 50 mer oligonucleotides in the MWG set. These additional genes serve as internal controls and allow cross-platform data analysis and harmonization, an on-going project of the C3B. The gene library was

| Control Genes                                      | Accession Number |
|---------------------------------------------------|------------------|
| GAPDH 5′                                           | M33197           |
| GAPDH 3′                                           | M33197           |
| Beta Actin 5′                                      | X00351           |
| Beta Actin 3′                                      | X00351           |
| ISGF 5′                                            | M97935           |
| ISGF 3′                                            | M97935           |
| Bio B                                              | J04422           |
| Bio C                                              | J04423           |
| Bio D                                              | J04424           |
| Thr C                                              | X04603           |
| Phe B                                              | M24537           |
| Phosphofructokinase, platelet                      | M64784           |
| Asparagine synthetase                              | M27396           |
| Aldolase A, fructose-bisphosphate                  | M11560           |
| Phosphoglycerate mutase 1 (brain)                  | XM_083842        |
| Glucose-6-phosphate dehydrogenase                  | M12996           |
| Ribosomal protein S3                               | AB061838         |
| Non-POU-domain-containing, octamer-binding         | XM_088688        |
| Ribosomal protein s27a                             | NM_002954        |
| Lactate dehydrogenase A                            | NM_005566        |
| Phosphoglycerate kinase 1 G                        | NM_000291        |
| Mitochondrial ribosomal protein L19                | NM_014763        |
| Rho GDP dissociation inhibitor (GDI) alpha         | AA453756         |
| Lactate dehydrogenase A                            | NM_005566        |
| Beta-2-microglobulin                               | NM_004048        |
| Phosphofructokinase, platelet                      | M64784           |
| Aldolase A, fructose-bisphosphate                  | M11560           |
| Ribosomal protein S27a                             | NM_002954        |
further supplemented with 78 custom-designed 50 mer oligos corresponding to genes previously documented in the literature as differentially expressed in brain tumors but which were not in the MWG library. The complete list of gene specific probes is publicly available (44).

The C3B oligo library was printed on γ-glycidoxy-modified (3-glycidoxypropyltrimethoxysilane; 0.1 wt% in anhydrous toluene for 30 min at 42°C then cured for 20 min at 110°C) 1.0’ × 3.0’ borosilicate glass microscope slides (31, 39) using a Cartesian PixSys 5500 Microarrayer. The arrays were produced by contact printing with a 4 × 2 pin arrangement using eight Parallel Synthesis spotting pins (Parallel Synthesis, Santa Clara, CA). The arrays were printed at room temperature under an air atmosphere of 50% relative humidity. The spotting buffer (pH = 5.2) was composed of 25 mM oligo in 1.5 X SSC and 0.75 M betaine [Diehl, 2001 #151]. The C3B10KO has a total of 10,584 independent genes that were spotted in duplicate creating an array with 21,168 total features divided into 4 × 12 (48) subgrids of 21 × 21 (441) spots (Fig. 14.1). The C3B10KO oligomicroarray has been the basis for cross-platform performance comparisons (31) for the development of cross-platform correlation estimates (32) and is intended to reveal patterns of gene expression to enable retrospective genetic class comparison, class delineation, and class prediction using differential gene expression profiling of IRB-approved banked and acquired tissues.

A parallel effort at the C3B has been the development of impedimetric and amperometric DNA hybridization detection platforms consisting of microlitho-

---

**Fig. 14.1** Schematic illustration of the layout of the C3B10KO oligo array showing the 4 × 12 (48) sub-grids, each with 21 × 21 (441) spots, including the various controls that resulted in the inclusion of 10,584 independent genes, spotted in duplicate, and creating an array with 21,168 total features
graphically fabricated interdigitated microsensor electrodes and microdisc array electrodes (45). These approaches focus on devising means to directly detect the hybridization of DNA on a multielement microelectrode device that will serve as the detector in a fully integrated molecular diagnostics system (46). The beBiochip-32™ and beBiochip-64™ consist of 32 regions of interdigitation and 64 microdisc pads that comprise 32- and 64-element arrays, respectively (Fig. 14.2). The beBiochip-32 functions by impedimetric detection of nanoparticle labeled and unlabeled DNA hybridization whereas the beBiochip-64 allows amperometric detection of redox-labeled DNA hybridization reactions. Fig. 14.2a shows an impedimetric array of 10 opposing fingers, each 2 microns wide and 3 mm long and separated by 1 micron spaces. Fig. 14.2b shows a voltammetric or amperometric sensor element comprising a hexagonal close-packed arrangement of microdiscs in the form of a microdisc electrode array, a large area (100 times the area of the microdisc working electrode) counter electrode, and a reference electrode.

The electrodes of this three-electrode electrochemical cell-on-a-chip were similarly fabricated from 100nm magnetron sputter-deposited gold or e-gun deposited platinum on a 10 nm titanium/tungsten adhesion promoting layer. The substrate used was either oxidized silicon with a minimum 100 nm of thermally grown oxide or the highly polished, electronics-grade, Schott D262 borosilicate glass. The microdisc array working electrode comprises a fluoro-etched opening through a 0.5 mm thick silicon nitride (Si₃N₄) layer that was deposited onto the previously patterned noble metal beneath. Collectively these biochips allow: (1) label-free impedimetric detection of DNA hybridization, (2) enhanced impedimetric detection using colloidal gold nanoparticles as labels on reverse-transcribed mRNA, and (3) the use of an electroactive layer of poly(pyrrole-co-pyrrolyl butanate) to provide covalent attachment of DNA probes and enhanced redox detection sensitivity with electroactive labels such as ferrocene. Significant changes in electrochemical impedance values (both real

![Fig. 14.2 Schematic illustration of a microfabricated multi-element array; a) comprising 32 interdigitated microsensor electrodes, and b) comprising 64 independently addressable microdisc voltammetric electrodes. Each device shows the large area counter electrode (middle) and the reference electrode as a band around the counter electrode.](image-url)
and imaginary components) (11% increase in impedance modulus at 120 Hz) have been detected after hybridization of covalently immobilized oligonucleotide probes to their complement (47).

Fig. 14.3a shows an impedimetric sensor element comprising a circumferentially arranged interdigitated microsensor electrode array, a large area (100 times the area of the interdigitated working electrodes) counterelectrode, and a reference electrode. The electrodes of this four-electrode electrochemical cell-on-a-chip were fabricated from 100 nm magnetron sputter-deposited gold or e-gun deposited platinum on a 10 nm titanium/tungsten adhesion promoting layer. The substrate used was either oxidized silicon with a minimum 100 nm of thermally grown oxide or the highly polished, electronics-grade, Schott D262 borosilicate glass. These independently addressable interdigitated electrodes could be used for two-electrode electrical impedance or in combination with the reference electrode, in three-electrode electrochemical impedance.

Finally, the two interdigitated electrodes could be shorted and used as a single working electrode within which the large area counterelectrode supports the electrochemical oxidation or reduction of appropriate electroactive species. As an example, electroconductive polymers that were grown by electropolymerization onto each region of interdigitation, was accomplished in this format. Finally, a reference electrode of silver/silver chloride accompanied each sensor element of the device and this was prepared by silverization of the gold or platinum layer of that electrode. This reference electrode provided the reference potential for the electrochemical impedance or amperometric determination of each multiplexed sensor element of the array. Fig. 14.3b shows the modified sensor element following silverization of the reference electrode. Here the bright silver prior to chloridization is shown. Fig. 14.3c shows
the modified sensor element following electropolymerization of a layer (exaggerated) of polypyrrole onto the region of interdigitation (48). Thin films of polypyrrole provide the anchorage chemistry for the covalent coupling of oligonucleotides.

**Fig. 14.4** shows a voltammetric or amperometric sensor element comprising a hexagonal close-packed arrangement of microdiscs in the form of a microdisc electrode array, a large area (100 times the area of the microdisc working electrode) counterelectrode and a reference electrode. The microdiscs are not independently addressable but rather exploit ultramicroelectrode electrochemistry in improving sensitivity, dynamic range, and signal-to-noise performance for a single DNA probe. The electrode designs of **Figs. 14.3 and 14.4** may also be implemented as individual sensor elements in a microfluidic system. **Fig. 14.5** shows a pair

![Fig. 14.4](image)

Fig. 14.4  a) Schematic illustration of the microfabricated voltammetric or amperometric microdisc array sensor element showing the three electrodes of the electrochemical cell-on-a-chip. b) Optical micrograph (x 50) of the microfabricated voltammetric or amperometric microdisc array sensor element showing the materials of construction

![Fig. 14.5](image)

Fig. 14.5  Optical micrograph of a microfabricated, dual-channel, electrochemical cell-on-a-chip interdigitated microsensor electrode (ECC IME) device showing the fluid flow over the microelectrode arrays. A=C2W2, B=C2CE, C=C2W1, D=C1W1, E=C1CE, F=C1W2, G=REF. C2 is Cell 2, C1 is Cell 1, W is working electrode, CE is counter electrode and REF is reference electrode.
of the four-electrode sensory elements of Fig. 14.3 that has been fabricated onto a single 1 cm × 2 cm × 0.05 cm glass chip. Fig. 14.6 shows a pair of the three-electrode sensory elements of Fig. 14.4 that has been fabricated onto a single 4 mm × 2 mm × 0.5 mm glass chip. Both chips offer a dual channel or a ratiometric detection scheme.

### 14.6 Impact and Challenges for Biochip-Based Molecular Diagnostics

Biochips are expected to have the greatest impact in the molecular diagnostics market. Here the major challenges go well beyond the usual technological challenges of acceptable clinical detection limits, levels of sensitivity and specificity, dynamic range, repeatability and reproducibility, response time, and immunity from false positives and false negatives. Although important, these analytical parameters must be matched to the specifics of the assay that is targeted and to the decision context of the acquired data.

One additional major challenge is the selection of appropriate molecular targets that are decidedly linked to disease susceptibility or drug response. This should prima facie be the point of initiation of all research and development activities on diagnostic biochips. The second major challenge is to overcome the fact that a biochip diagnostic will simply be an additional format for delivering an assay that could otherwise be done using the same reagent set and assay approaches that are implemented on the chip. This is not a trivial matter and should not be overlooked. After all, identification of the molecular target as “appropriate” would have originated using lab-based assays. The biochip format must therefore offer some significant competitive advantage over its lab-based equivalent.
Areas of competitive advantage for diagnostic and prognostic biochips may include the following.

(i) **Higher throughput.** The biochip format, when automated, may permit the handling of many samples. This will reduce the overall cost per test performed. The consequence is that the platform migrates away from the point of concern and is then favored in the clinical or molecular diagnostics laboratory.

(ii) **Smaller sample volume.** The biochip format allows smaller sample volumes to be used. This is relevant when samples are available in very small quantities such as in some tumor biopsies.

(iii) **Integration of sample preparation.** Sample preparation and/or workup are a major source of error and cost in many molecular diagnostics. The ability to integrate these unit operations (such as cell sorting, lysis, protein separation, PCR) onto the same biochip platform is a major advantage. Although this does not save time, it can limit the need for operator involvement and allow the diagnostic test to migrate closer to the site of interest (near patient: physician’s office or bedside).

Additional major challenges include the size of the market, access via distribution channels, and payer issues. The last is likely to be the most important forester of what could potentially have the largest impact on the way health care is provided.

The impact of diagnostic biochips in molecular diagnostics must await the results of further research and targeted product development. Several companies, including Millennium Predictive Medicine, Abbott Diagnostics, Bayer, and ABTECH Scientific are actively pursuing such diagnostic products. Once these products are developed, validated, clinically tried, and approved by the FDA, they must be sold to a traditionally conservative medical community. This community has been slow to adopt new diagnostic paradigms which are due, in part, to many overhyped claims for other approaches in the past. Genomics-derived molecular diagnostics are likely to have a significant impact on the market by the end of the current decade.

### 14.7 Issues Related to Diagnostic Biochips

A burgeoning issue, unique to biochip technologies, is the plurality of technological approaches being researched, developed, and commercialized. Market forces (capital, speed to market, product positioning) rather than policy requirements will drive end-user choices among the several dozen competing biochip technologies available. Each of these requires sophisticated software programs and powerful computing capabilities in the design of chip functionality as well as in the capture, processing, and analysis of the massive amounts of data they will generate. As an example, the absence of a standard format for comparing and transferring microarray data has produced proposals to create a standard “language” to facilitate the sharing of data among scientists (49). Open structure databases such as Gene X, may emerge with the capa-
bility to accept Affymetrix GeneChip® data, cDNA data, and spotted oligonucleotide microarray data. When successfully integrated with clinical databases, these will be the forerunners of the dedicated systems to be used in clinical diagnostics.

A second major issue for the diagnostic biochip is the need to define an appropriate complement of genes and a large number of alleles (various forms of the same gene) that are appropriate for the decision-making requirements in a given clinical context. This creates pressures from two opposing sides: the need to have licensed access to a sufficiently large number of genes to achieve the diagnostic purpose and the competing need to limit the total number of genes to make the diagnostic commercially viable. To eliminate this downward pressure, patent licensing strategies may evolve to facilitate the exchange of rights between the competing entities who “own” the rights in the legion of genes utilized on the biochip platforms. “An equitable and streamlined mechanism for licensing genes and alleles would help to promote continued research, development and commercialization” (50). One proposal that has been advanced has been to “create a compulsory licensing scheme for DNA sequences used on gene chips, modeling it on the statutory licensing arrangement for music under the Copyright Act” (51).

A third and confounding issue pits genetic screening using genomic microarrays against the more targeted diagnostic biochips. The likely economics of multiple genetic tests makes it preferable to screen for many diseases and susceptibilities at once using genomic microarrays. Although screening may have benefits, it raises several questions:

(i) Are there adequate interventions or therapies revealed by the screen?
(ii) Are the privacy rights of patients adequately protected?
(iii) Are genetic counselors suitably prepared to guide the interpretation of the myriad risk-based assessments associated with a broad genomic screen?
(iv) Can informed consent be obtained given the broad range of possible outcomes of the screen?

Some of these questions may be adequately addressed with established procedures such as institutional review boards (IRBs), tiered informed consent, recontact or follow-up protocols, and anonymizing or keycoding of patient data. The potential for economies of scale in the screening of patients must be balanced by the patient’s right to privacy protection. The potential for a full genomic scan to reveal not just what is currently known about an individual, but also, once entered into a database, that which may be known in the future, not just about an individual but also about their progeny, siblings, and lineage, must dampen enthusiasm for such a genomic screen. The solution is the development of specific gene chips, diagnostic and prognostic biochips that target specific disease states or genetic conditions. It is evident that the current genomic gene chips will be replaced by more dedicated Oncochips®, arthritis chips, and so on, which assure individual protection and meet the rigors required for IRB approval.

A fourth issue related to diagnostic biochip testing arises with the accumulation of detailed health information in databanks. “Such databanks are powerful and valuable tools to help understand and counteract disease processes” but raise questions
of equity of access and parity of use in structuring inquiry for societal benefits (52). Of course, such issues must be balanced by the intellectual property rights of those who invest in the development of such databases.

### 14.8 Concluding Remarks

The diagnostic biochip will emerge as a highly quantitative, clinically focused molecular diagnostic tool for the modern diagnostics laboratory. However, the road to this destination is filled with regulatory requirements and societal concerns, to which the technological and practitioner communities must be cognizant and responsive. Furthermore, the technology, in its current format, is labor-intensive and quite expensive. There is need for alternative formats that focus on specific diagnostic or prognostic applications within targeted disease states. Better methods of manufacture and detection are being developed, including direct detection of hybridization by electric current, based on the fact that single-stranded DNA conducts electrons at a different rate than double-stranded DNA. In the near future, nucleic acid nanotechnology may prove very useful in DNA diagnostics. Research has led to advanced nucleic acid nanostructures and devices, semisynthetic DNA–protein conjugates, and efficient assembly of individual oligonucleotide-functionalized nanoparticles in two- and three-dimensional networks (53). Some of these assemblies have already proven useful for diagnostic purposes (54, 55) and the construction of nanometer-sized DNA-based nanowires has been achieved (56). The latter have been incorporated into a DNA-based two-dimensional network of functional scaffolds for protein arrays. It will only be a short time before similar DNA arrays are developed.

**Acknowledgments**  This work was supported by the industrial consortium of the Center for Bioelectronics, Biosensors and Biochips and by the Commonwealth Technologies Research Fund (CTRF) Grant SE2002-02. This chapter is dedicated to the memory of Dr. Anil K. Deisingh who passed away on October 21st, 2005 and was at the time a member of the technical staff of the Analytical Chemistry and Microbiology Services Unit, Caribbean Industrial Research Institute, University of the West Indies Campus, St. Augustine, Republic of Trinidad & Tobago.

**References**

1. Guiseppi-Elie, A. (2003) Biochip platforms for DNA diagnostics. *Business Briefing: PharmaTech*, London, World Markets Research Centre, p. 87.
2. Persidis, A. (1998) *Nature Biotechnology*, 16, 981–983.
3. Proudnikov, D., Timofeev, E., and Mirzabekov, A. (1998) Immobilization of DNA in polyacrylamide gel for the manufacture of DNA and DNA–oligonucleotide microchips. *Analytical Biochemistry* 259(1), 34–41. Llewellyn, B., Lebed, J., and Chik, V. (2002), [www.promega.com/geneticidproc/ussymplproc/content/llewellyn.pdf](http://www.promega.com/geneticidproc/ussymplproc/content/llewellyn.pdf).
4. Seetharaman, S., Zivarts, M., Sundarsan, N., and Breaker, R.R. (2001) Nature Biotechnology, 19, 336–341.
5. Wagner, P. and Kim, R. (May 2002) www.currentdrugdiscovery.com, pp. 23–28.
6. Gad-el-Hak, Mohamed, Ed. (2001) The MEMS Handbook. CRC Press, Boca Raton, FL. The MEMS Exchange (2002), http://www.mems-exchange.org/MEMS/what-is.html.
7. Christian, G.D. (1994) Analytical Chemistry, 5th ed., Chapter 13. Wiley, New York, 384–397.
8. Cunningham, A.J. (1998) Introduction to Bioanalytical Sensors, Techniques in Analytical Chemistry. John Wiley & Sons, Inc. New York.
9. A. Guiseppi-Elie and L. Lingerfelt “Impedimetric Detection of DNA Hybridization: Towards Near Patient DNA Diagnostics“ In Immobilization of DNA on Chips I (2005); Christine Wittmann, Ed.; Topics in Current Chemistry Vol. 260, Springer Berlin, Heidelberg, pp 161–186.
10. Turner, A.P.F. and Newman, J.D. (1998) Biosensors for Food Analysis, A.O. Scott (Ed.). Royal Society of Chemistry, Cambridge, pp. 1–10.
11. Steemers, F.J. and Walt, D.R. (1999) Multi-analyte sensing: from site-selective deposition to randomly-ordered addressable optical sensors. Microchimica Acta, 131 (1–2): 99–105.
12. Brogan, K.L. and Walt, D.R. (2005) Optical fiber-based sensors: Application to chemical biology. Current Opinion in Chem. Biology, 9, 494–500.
13. Hall, E.A.H. (1990) Biosensors. Open University Press, Milton Keynes, UK.
14. Blohm, D.H. and Guiseppi-Elie, A. (2001) Current Opinion in Biotechnology, 12, 41–47.
15. Wang, J. (2000) Nucleic Acids Research, 28 (16), 3011–3016.
16. Schena, M. (2002) Microarray Analysis. Wiley-Liss, New York.
17. NCBI (2003) http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html (accessed on May 5th, 2008).
18. Draghici, S. (2003) Data Analysis Tools for DNA Microarrays. Chapman & Hall/CRC, Boca Raton, FL.
19. Vahey, M., Nau, M.E., Barrick, S., Cooley, J.D., Sawyer, R., Sleeker, A.A., Vickerman, P., Bloor, S., Larder, B., Michael, N.L., and Wegner, S.A. (1999) Journal of Clinical Microbiology, 37, 2533–2537.
20. Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Koh, M.L., Downing, J.R., Caliguiari, M.A., Bloomfield, C.D., and Lander, E.S. (1999) Science, 286, 531–537.
21. Taylor, S., Smith, S., Windle, B., and Guiseppi-Elie, A. (2003) Impact of surface chemistry and blocking strategies in DNA microarrays. Nucleic Acids Research, 31(16), e87.
22. Windle, B. and Guiseppi-Elie, A. (2003) Microarrays and gene expression profiling applied to drug research. In Burger’s Medicinal Chemistry, 6th ed., D.J. Abraham (Ed.). Wiley, New York.
23. Willis, R.C. (2003), Monitoring microbes. Modern Drug Discovery, January, 16–21.
24. Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J., and Harel, J. (2003) Journal of Clinical Microbiology, 41, 2113–2125.
25. Volokhov, D., Rasooly, A., Chumakov, K., and Chizhikov, V. (2002) Journal of Clinical Microbiology, 40, 4720–4728.
26. Hanna, G.J., Johnson, V.A., Kuritzkes, D.R., Richman, D.D., Martinex-Picado, J., Sutton, L., Hazelwood, J.D., and D’Aquila, R.T. (2000) Journal of Clinical Microbiology, 38, 2715–2721.
27. Li, J., Chen, S., and Evans, D.H. (2001) Journal of Clinical Microbiology, 39, 696–704.
28. Chizhikov, V., Wagner, M., Ivshina, A., Hoshino, Y., Kapikian, A.Z., and Chumakov, K. (2002) Journal of Clinical Microbiology, 40, 2398–2407.
29. Wang, D., Coscoy, L., Zylberberg, M., Avila, P.C., Boushey, H.A., Ganem, D., and DeRisi, J.L. (2002) Proceedings of the National Academy of Sciences of the USA, 99, 15687–15692.
30. Higgins, J.P.T., Shinghal, R., Gill, H., Reese, J.H., Terris, M., Cohen, R.J., Fero, M., Pollack, J.R., vandeRijn, M., and Brooks, J.D. (2003) American Journal of Pathology, 162(3), 925–932.
31. Archer, K.J., Dumur, C.I., Scott Taylor, G., Chaplin, M.D., Guiseppi-Elie, A., Buck, G., Grant, G., Ferreira-Gonzalez, A., and Garrett, C. (2008) A disattenuated correlation estimate when variables are measured with error: Illustration estimating cross-platform correlations. *Statistics in Medicine*, 27(7), 1026–1039.

32. Archer, K.J., Dumur, C.I., Taylor, G.S., Chaplin, M.D. Guiseppi-Elie, A., Grant, G., Ferreira-Gonzalez, A., and Garrett, C. (2007) Application of a correlation correction factor in a microarray cross-platform reproducibility study. *BMC Bioinformatics*, 8, 447.

33. Martinez, J.M., Aragon, A.D., Rodriguez, A.L., Weber, J.M., Timlin, J.A., Sinclair, M.B., Haaland, D.M., and Werner-Washburne, M. (2003) *Nucleic Acid Research*, 31(4), e18.

34. van Brunt, J. (2003) *Signals Magazine* (online), 25th April 2003, http://www.signalsmag.com/signalsmag.nsf/0/6D4F7034D24D25988256D130011E957?Open.

35. SNP Consortium (2001) http://www.snp.cshl.org/about/2001_TSC_project.overview.shtml. (accessed on May 5th, 2008)

36. NIH New Advisory (October 2002) http://www.genome.gov/page.cfm?pageID=10005336. (accessed on May 5th, 2008)

37. McNeely, G. (May 5th, 2003) *Small Times On-Line IEEE* http://www.smalltimes.com/articles/article_display.cfm?Section=ARCHI&C=Profi&ARTICLE_ID=268701&p=109 (accessed on May 5th, 2008)

38. Gupta, R., Kim, J.P., Spiegel, J., and Ferguson, S.M (2004) Developing products for personalized medicine: NIH research tools policy applications. *Personalized Medicine* 1(1): 115–124.

39. Ginsburg, G.S. and Angrist, M. (2006) The future may be closer than you think: a response from the Personalized Medicine Coalition to the Royal Society’s report on personalized medicine. *Personalized Medicine*, 3(2): 119–123.

40. Giles, F.J., Cortes, J.E., and Kantarjian, H.M. (2005) Targeting the kinase activity of the BCR-ABL fusion protein in patients with chronic myelogenous leukemia. *Current Molecular Medicine* 5(7):615–623.

41. Druker, B.J., Talpaz, M., Resta, D.J., Peng, B., Buchdunger, E., and Ford, J.M. (2001) *The New England Journal of Medicine*, 344(14), 1031–1037.

42. Case-Green, S.C., Mir, K.U, Pritchard, C.E., Southernn, E.M. (1998) Analyzing genetic information with DNA arrays. *Current Opinion in Biotechnology* 2, 404–410.

43. Anthony Guiseppi-Elie, Scott Taylor, Louise Lingerfelt, Chris Nixon, Ryan Georgiana, Joy Kim, Stephanie Smith, Brad Mangrum and Nicholas Farell “Studies of the Interaction of Platinum Drugs with DNA Using Oligonucleotide Microarrays” *Macromolecular Symposia* (2006) 235(1), 115–120

44. CTRF Consortium of Virginia http://www.ctrf-cagenomics.vcu.edu/publiclyavaildata.htm. (accessed on May 5th, 2008)

45. Lingerfelt, L., Karlinsky, J., Landers, J., and Guiseppi-Elie, A. (2008) Impedimetric detection for DNA hybridization within microfluidic biochips. In *Microchip-Based Assay Systems Methods in Molecular Biology*, Pierre N. Floriano, Ed.; Royal Society of Chemistry. Humana Press, NJ. vol. 385, Chapter 8, pp 103–120.

46. Katz, E. and Willner, I. (2003) Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: Routes to impedimetric immunosensors, DNA-Sensors, and enzyme biosensors. *Electroanalysis*, 15, 913–947.

47. Hang Tin, C. and Guiseppi-Elie, A. (2004) Frequency dependent and surface characterization of DNA immobilization and hybridization. *Biosensors and Bioelectronics* 19, 1537–1548.

48. Guiseppi-Elie, A., Brahim, S., and Wilson, A. (2007) Biosensors based on electrically conducting polymers. In *Handbook of Conducting Polymers: Conjugated Polymer Processing and Applications*; 3rd ed., T. Skotheim and J.R. Reynolds (Eds.). Taylor and Francis, New York. Chapter 12, pp. 12:1–12:45.

49. Davenport, R.J. (2001) Microarrays: Data standards on the horizon. *Science*, 292, 414–415.

50. Williams, E. (2002) *Gene Chips: Science and Policy Basics*, 2002 Virginia Biotechnology Summit and Governor’s Conference on Technology Transfer and University Research, McLean, Virginia, October 14–16.
51. Johns, D.J.; Brettwisch, R., and Lebovitz, R. (1991) Patenting DNA: Letter to the editor. *Science*, 254 (5036), 1276.

52. Williams, E.D. (2001) The policy and ethics of DNA chip technologies. In N. Fujiki, M. Sudo, and D.R.J. Macer (Eds.), *Bioethics and the Impact of Human Genome Research in the 21st Century: Pharmacogenomics, DNA polymorphism and Medical Genetics Services*, 104–109, Eubios Ethics Institute, Tsukuba, Japan.

53. Wengel, J. (2004) *Analyst*, 2, 277–280.

54. Niemeyer, C.M. (2002) The developments of semisynthetic DNA-protein conjugates. *Trends Biotechnol.*, 20, 395–401.

55. Niemeyer, C.M. (2002) Nanotechnology: Tools for the biomolecular engineer. *Science*, 297 (5578), 62–63.

56. Yan, H., Park, S.H., Finkelstein, G., Reif, J.H., and La Bean, T.H. (2003) *Science*, 301, 1882–1884.