Invited Paper

Research and development of biochip technologies in Taiwan

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ABSTRACT

Recent advancements in several genome-sequencing projects have stimulated an enormous interest in microarray DNA chip technology, especially in biomedical sciences and pharmaceutical industries. The DNA chips facilitated the miniaturization of conventional nucleic acid hybridizations, by either robotically spotting thousands of library cDNAs or *in situ* synthesis of high-density oligonucleotides onto solid supports. These innovations have found a wide range of applications in molecular biology, especially in studying gene expression and discovering new genes from the global view of genomic analysis. The research and development of this powerful tool has also received great attentions in Taiwan. In this paper, we report the current progresses of our DNA chip project, along with the current status of other biochip projects in Taiwan, such as protein chip, PCR chip, electrophoresis chip, olfactory chip, etc. The new development of biochip technologies integrates the biotechnology with the semiconductor processing, the micro-electro-mechanical, optoelectronic, and digital signal processing technologies. Most of these biochip technologies utilize optical detection methods for data acquisition and analysis. The strengths and advantages of different approaches are compared and discussed in this report.

Keywords: Biochip, genome, micro-strip, peptide nucleic acid, protein, microarray, optical detection

1. INTRODUCTION

Biochip technologies have recently received great attentions in Taiwan. There were 10 biochip-related research projects granted by the National Science Council (NSC) in the year of 1999. In the year of 2000, about 40 proposals were submitted to the NSC (besides many industry-oriented projects supported by either private or other government agencies) for the research and development (R&D) in the DNA chips, the protein chips, the micro-fluidic chips, and other biosensors. (See Table 1.) The major driving force for such a sharp rise in R&D of biochip technologies comes from the impact of genome sequencing projects.[1] The successful advancements in several international collaborations of large scale sequencing of whole genome, either of human or model organisms, have generated a tremendous amount of information, which is extremely precious (from both the scientific and the economic points of view) in biomedical science and pharmaceutical industries. A very efficient approach to utilize the unprecedented genomic information is the miniaturization of conventional biotechnology, integrated with the semiconductor processing, the micro-electro-mechanical, optoelectronic, and digital signal processing technologies. Since Taiwan has been very successful in these chip-based industries, researchers in Taiwan are motivated to take advantages of the resources and the lessons learned from these chip-based industries, and to apply them in the frontiers of the biochip technologies.

In a generic sense, any device or component incorporating biological or organic materials, either extracted from organisms or synthesized in a laboratory, on a solid substrate can be regarded as a biochip. From the practical point of view and from the analogy taken from the IC chip, the term "biochip", however, often implies some miniaturization, and the possibility of low-cost, high-throughput mass production. Some of the examples that meet the qualification stated above include the DNA chip (or the gene chip),[2] the protein chip,[3] the PCR (polymerase chain reaction) chip,[4] the capillary electrophoresis chip,[5] and the biosensor chip.[6] In this paper, we give a brief overview of the R&D status of various biochip projects in Taiwan.

2. THE DNA CHIP

2.1. Working principle

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A DNA chip refers to a two-dimensional array of small reaction cells (each on the order of 100 μm × 100 μm) fabricated on a solid substrate. The solid substrate can be a silicon wafer, or a thin sheet of glass or plastic, or a nylon membrane. In each reaction cell, trillions of polymeric molecules of a specific sequence of single-stranded DNA are immobilized as illustrated in Fig. 1. The DNA molecules can be either short sequence of oligonucleotides (~20 to 25 bases) or longer fragments (~1,000 to 3,000 bases) of complementary DNA (cDNA).[7] The specific sequence of oligonucleotides or fragments in each cell is pre-selected or designed based on the intended application. The known sequences of single-stranded DNA immobilized on the substrate are often called the probes. When unknown fragments of single-stranded DNA samples (often called the target) react (or hybridize) with the probes on the chip, double-stranded DNA fragments are formed where the target and the probe are complementary according to the base pairing rule (A paired with T, and G paired with C). To facilitate the analysis of the hybridized chip, the target samples are often labeled with a tag, such as a fluorescent, a dye, or an enzyme. When the targets contain more than one type of sample, each is labeled with its own distinguishable tag. Depending on the size of the array, the DNA chip described above provides a platform where the unknown targets can potentially be identified with very high speed and high throughput by matching with tens of thousands of different types of probes via hybridization in parallel. In the DNA chip using oligonucleotides as probes, the formation of the double-stranded DNA by the base-pairing rule is so specific that under favorable conditions even a single base-pair mismatch can be detected and identified.[8]

2.2. Fabrication techniques

DNA chips are often fabricated by one of the following popular techniques: (1) robotic spotting, which uses DNA fragments as probes,[9] (2) photolithography, which utilizes the synthesized oligonucleotides as probes.[10] In the first technique, all the DNA fragments to be immobilized on the substrate are pre-amplified (by polymerase chain reaction), and stored in a set of individual containers. A robotic arm with an array of tips is used to transfer the pre-amplified DNA fragments on to the solid substrate by first dipping into the containers and then touching the substrate. In the second approach, the desired sequences of oligonucleotides are synthesized in parallel, layer by layer, using photolithographic technology as illustrated in Fig. 2.[11] In this approach, the substrate is first pre-coated with appropriate linkers and protectors. An ultraviolet (UV) light is used to illuminate the substrate through a photolithographic mask to remove the protecting group at specific sites where one particular type of base (say A, for example) is immobilized (via proper biochemical reactions) as illustrated in Fig. 2(a). Likewise, a second mask is then used to immobilize another kind of base (T, for example) at other selected sites as in Fig. 2(b). Four photolithographic masks are thus needed to fill the first layer with all the four types of bases. Successive rounds of de-protection (via UV illumination) and immobilization (via biochemical reactions) are carried out to synthesize the desired sequences of bases at each site, spatially addressable, layer by layer, in parallel. For a sequence of N bases, the total number of photo masks required is 4N, in general. This approach is hence more suited for the synthesis of short sequences of bases. Typically, the length of oligonucleotide is limited to 25 bases or less. When the peptide nucleic acid (PNA)[12] is substituted for the oligonucleotide, the sequence of the probe can be reduced (say, from 25 bases to about 15 bases) with the same specificity in hybridization. This alternative can greatly reduce the cost of photo masks and chemicals for the syntheses, and save the processing time. Some advantages and disadvantages of the two fabrication techniques are listed in Table 2.

The DNA chip designed and currently being developed by our research group[13] includes lines of electrodes (in the form of micro-strips) connecting arrays of reaction cells (size = 50μm x 50μm each, center-to-center spacing = 75μm, total number of cells = 100x100) as illustrated in Fig. 3. During the reaction (or hybridization) cycle, an appropriate positive voltage can be applied to selected strip of sites to concentrate the negatively charged DNA targets to selected sites. After the hybridization, during the "wash" cycle, an appropriate negative voltage can be applied to the sites to drive away (or wash out) the mismatched DNA targets. This technique can potentially reduce the hybridization time from hours to minutes. The probes made of PNA are particularly suitable for such kind of design since the PNA molecules are neutral and therefore not affected by the micro-strip electrodes.

2.3. Signal readout from hybridized DNA chips

The next step after the hybridization process is the signal detection or readout to determine the sites where the sequence of the unknown DNA targets complement (or match) that of the probes and stick to the site via the formation of double-stranded DNA molecules. The particular choice of signal detection techniques goes hand in hand with the choice of the tag attached to the DNA target molecules. For example, if one chooses to use fluorescence signal for detection, appropriate fluorescent molecules will be used as the tags. The signal readout system will then consist of an appropriate light source for

excitation, a photo-multiplier tube or a CCD camera (with an appropriate filter) for the detection of the fluorescence emission, and a personal computer for data acquisition and post processing. A schematic simplified diagram of such a signal detection system is shown in Fig. 4. Recently, we have developed a multi-point excitation and CCD based imaging system for high-throughput fluorescence detection of biochip microarrays. Details of the development and the characterization of the system will be reported in a separate paper. Although one can, in principle, integrate either the CCD or the CMOS imager with the DNA micro-array on the same silicon wafer chip, such an approach will unavoidably increase the complexity, and hence the cost, of the fabrication.[14] Unless the fabricated chips are re-usable, such an approach may not be competitive from an economic point of view. Many commercial optical readout systems have been developed in recent years.[15] An alternative optical technique for signal detection is to use dye molecules as the tags and use colorimetry for signal detection and discrimination.[16] Techniques, other than optical, such as radio-isotopic, electronic, or time-of-flight mass spectrometric, have also been investigated.

3. THE PROTEIN CHIP

3.1. Working principle

A protein chip typically consists of an array of spots (each on the order of 100 μm up to 1 mm in diameter) fabricated on a conducting support.[17] Each spot is designed to capture specific proteins of interest from the test sample, with an affinity capture surface, by either a chemical (ionic, hydrophobic, hydrophilic) or a biochemical (antibody, receptor) mechanism depending on the intended application. When the protein sample from cell lysate, serum, or urine is dispensed onto the surface of a protein chip, proteins of interest will be captured on spots according to affinity binding. After removing unbound proteins and interfering substances, the purified proteins on each spot are examined through a laser-induced process, called matrix-assisted (or surface enhanced) laser desorption/ionization (MALDI or SELDI),[3] followed by a mass analysis using time-of-flight mass spectroscopy (TOF-MS).[18] The molecular weights of the sample proteins captured or components of sample proteins (epitope of an antigen) can then be rapidly determined from spot to spot. The protein chip technology can play an important role in the discovery of disease biomarkers or the diagnostics of specific disease when the associated biomarkers are already known. It is expected to have a direct impact on drug discovery. A schematic illustration of the working principle of a protein chip is shown in Fig. 5.

3.2. Development of protein chip techniques

The techniques involved in a protein chip system are much less developed in Taiwan than those of a DNA chip. The National Health Research Institute has played a major role in the promotion of the R&D of protein chips. The protein chips and the related technologies will certainly attract the attention of more scientists in Taiwan in the near future. The number of researchers working in this area is expected to increase significantly in the next few years.

4. THE PCR(POLYMERASE CHAIN REACTION) CHIP

In many applications, the DNA samples to be tested (or identified) need to be purified and amplified to increase its amount by orders of magnitude to a detectable level. The amplification of DNA samples is often accomplished by polymerase chain reaction (PCR)[19] in which a minute amount of DNA molecules are repeatedly thermal cycled through a sequence of temperature stages as illustrated in Fig. 6. The amount of the DNA molecules is doubled at the end of each cycle. Each cycle consists of denaturation (the splitting of each double-stranded DNA molecule into two complementary single strands), primer annealing, and primer extension (the reconstruction of double-stranded DNA molecules from single-stranded components through the DNA polymerase and the proper reagents at the proper temperatures). The conventional PCR system can also be miniaturized and fabricated on a chip by micro-machining and microfluidic technologies.[4] A group of researchers at the National Cheng Kung University has successfully developed a micro-PCR chip, which can rapidly amplify cDNA of Hepatitis C virus for 30 cycles in 30 minutes, compared to 5.5 hours with traditional PCR equipment.[20]

5. THE CE(CAPILLARY ELECTROPHORESIS) CHIP

Capillary electrophoresis has been accepted as an extremely efficient technique for the separation of small sample volume in low concentration. The time scale of a complete run of capillary electrophoresis can be less than 10% of that needed for the conventional gel electrophoresis. A team at the National Cheng Kung University has successfully developed a microchip of capillary electrophoresis that can separate and identify in less than 3 minutes DNA fragments, ϕX174RF DNA digested by

the restriction enzyme *Hae*III.[21] The microfluidic chips were fabricated on poly(methyl methacrylate) (PMMA) substrate using two small-diameter (79 μm) wires to create a cross impression while the substrate was softened by low-temperature heating. (This CE chip can now be routinely produced using etched quartz template.[22]) The schematic illustration is shown in Fig. 7. The resulting channels have a rounded shape and are 75 μm deep. The horizontal channel is 20 mm long and serves as the loading channel for the analyte; the vertical channel is 50 mm long and used as the separation channel. The electric field applied to each channel is maintained at 300 V/cm or less to prevent Joule heating. In a typical test run, DNA samples at a concentration of about 1 μg/ml were first injected into the loading channel and the electric field was applied to the loading channel. When the peak of sample arrived at the junction of two channels, the applied electric field was switched from the loading channel to the separation channel. The detection point was 30 mm away from the junction. It took less than 3 minutes to run and identify all 11 fragments of ϕX174RF digest as illustrated in Fig. 8. The performance of such a capillary electrophoresis chip proves to be reliable and competitive to other designs.

6. THE BIOSENSOR CHIP

One of the most significant biosensor projects in Taiwan is the R&D of electronic noses (also known as the olfactory chips). An electronic nose is a device that can detect and identify specific odorant molecules. It often consists of an array of chemical sensing elements and a pattern recognition system. The mammalian olfactory uses a variety of receptors to identify odor or volatile compound. The mechanism of identification of a specific odor is not a one-to-one mapping of one specific type of odorant molecules with one corresponding type of receptors. It is the collective set of receptors (responding with varying degrees to each type of odorant molecules) combined with pattern recognition that identifies each odor. A team at the National Dong Hwa University has successfully developed the olfactory chip.[23] This research group used piezoelectric quartz crystals (which resonate at precise frequencies) coated with selective coatings (peptide derivatives of the various mammalian olfactory receptors) to adsorb species of molecules. The adsorbed molecules increase the mass of the sensor; and thus the resonance frequency of the piezoelectric quartz crystal is changed. By measuring the shift in resonance frequency, the concentration of odorant can be derived. The advantages of this approach include high selectivity, high sensitivity, and good reproducibility. An array of multi-sensors has been assembled for pattern recognition, using numerical taxonomy or artificial neural networks. The working principle of an electronic nose is illustrated in Fig. 9. This olfactory chip system has recently been developed for diagnostics of dengue fever, a disease propagated by Aedes mosquitoes, and is expected to be commercialized soon.

7. SUMMARY

The efforts to develop various biochip technologies are definitely growing rapidly in Taiwan. The interdisciplinary nature of these novel technologies is obvious. The fabrication of biochips requires close collaboration of scientists and engineers from different disciplines such as photolithography, micro-electro-mechanical system, microfluidic technologies, and biochemistry. The choice of the specific DNA sequences on the chips is dictated by the target applications and may involve expertise from medical, pharmaceutical, or biological sciences. The technologies associated with detection and readout system may involve optics and opto-electronics, analytical chemistry, or semiconductor device physics. The processing, visualization, interpretation, and management of data require the collaborative effort of computer scientists, mathematicians, and the end users for whom the specific chips are designed. Hopefully, high quality and efficient products of biochip technologies will soon be available in Taiwan for research and commercial applications.

ACKNOWLEDGEMENT

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Table 1. Biochip-related projects in Taiwan.

	Number of projects	
	Year 1999 (granted by NSC)	Year 2000 (submitted to NSC)
DNA chips	9	16
Protein chips	0	7
Micro-fluidic chips	1	11
Biosensors	0	3
Total	10	37

Table 2. A comparison of some advantages and disadvantages of different techniques for DNA chip fabrication.

Techniques	Advantages	Disadvantages
Photolithography	• Versatile • High density • Large array size • Mass production	• High up-front cost • Time-consuming
Mechanical Micro-spotting	• Affordable • Simple	• Sample storage • Low throughput

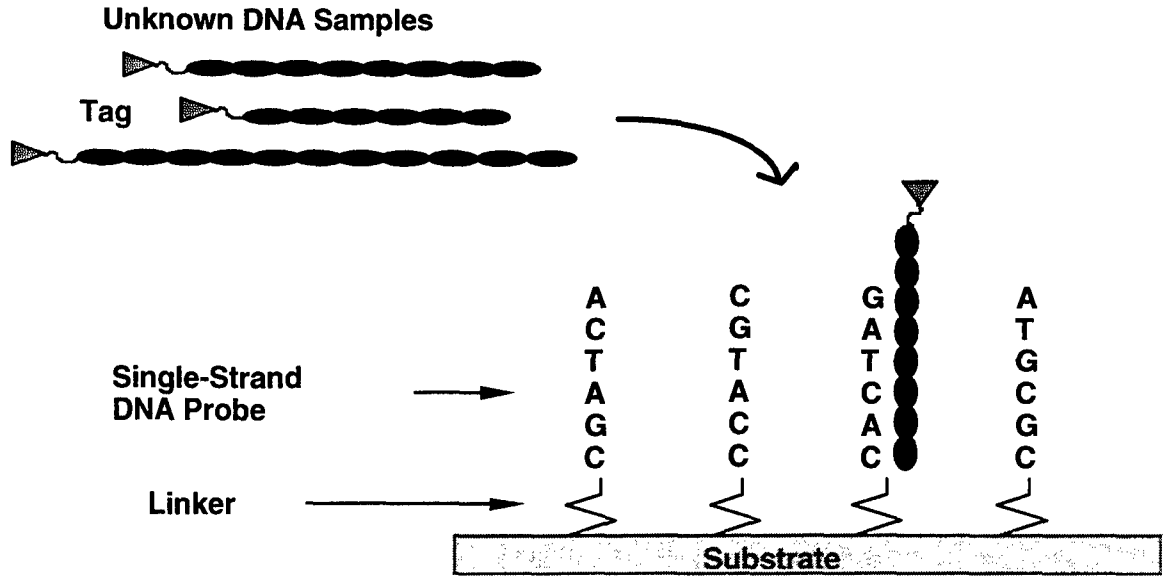

Fig. 1. A schematic illustration of the basic principle of a DNA microarray chip.

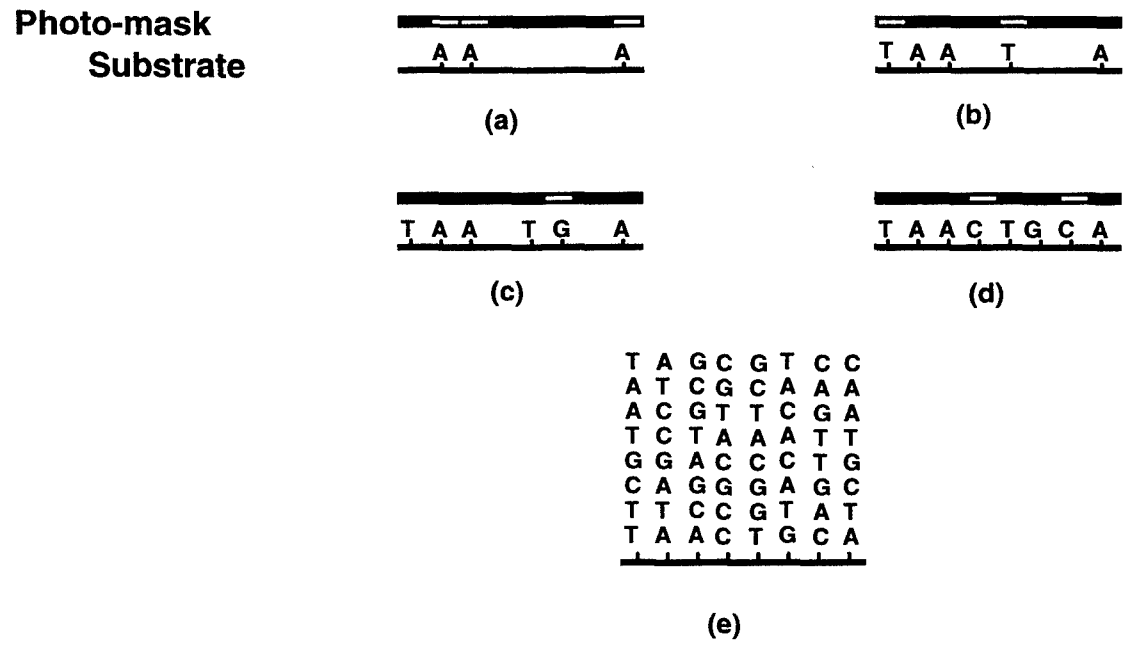

Fig. 2. *In situ* synthesis of DNA fragments (or sequence of oligonucleotides) by photolithographic technique.

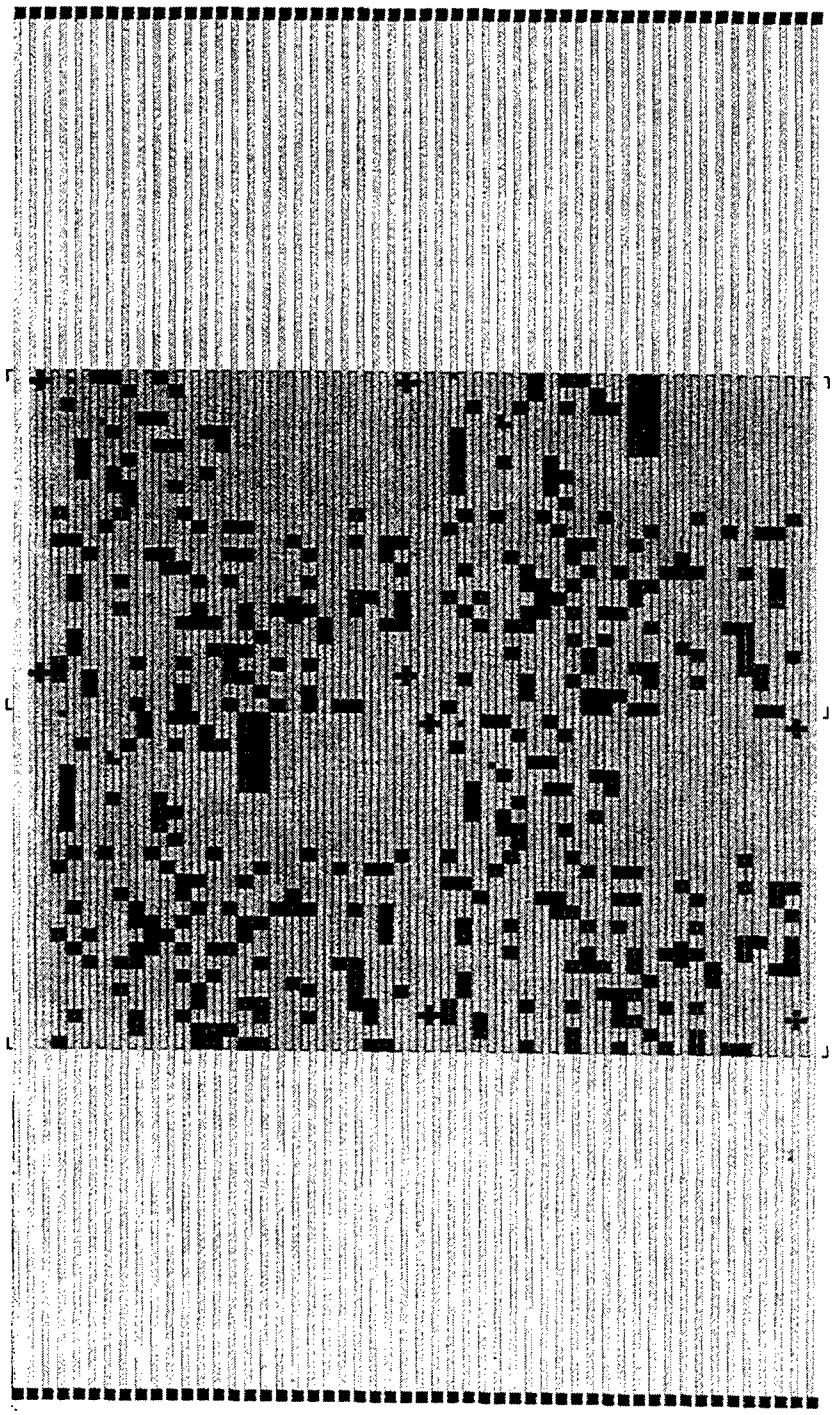

Fig. 3. A schematic illustration of a DNA chip including lines of electrodes (in the form of micro-strips) in concomitant with the array of reaction cells.

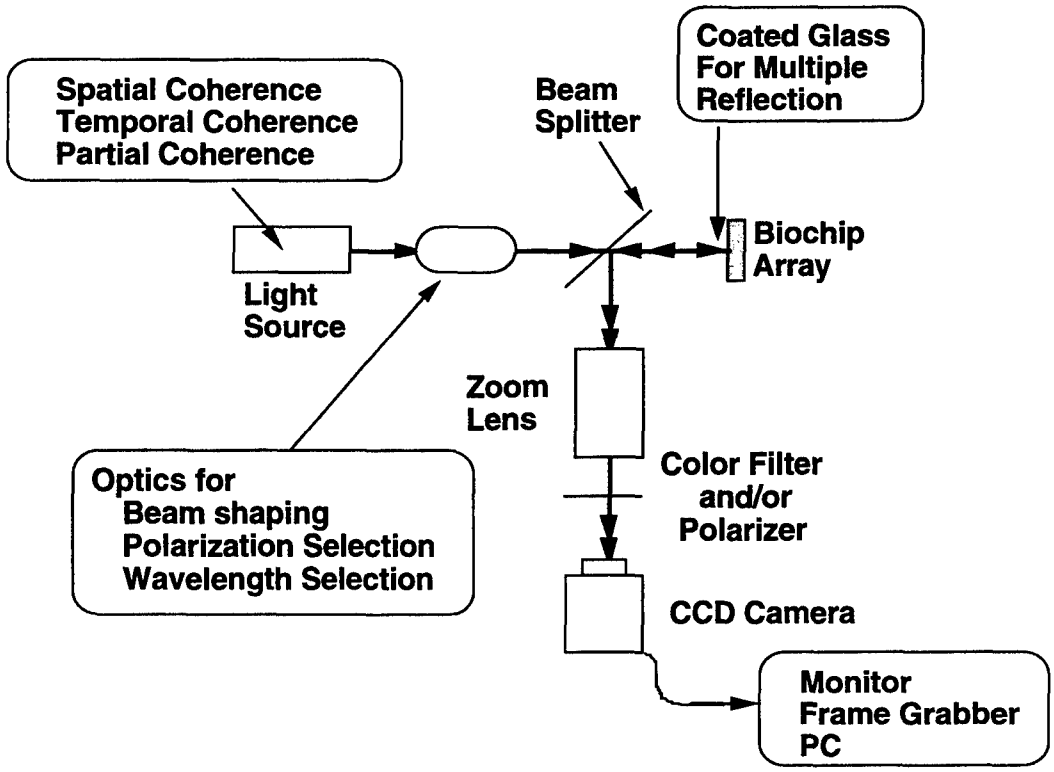

Fig. 4. A schematic illustration of a CCD-based imaging system for biochip signal detection.

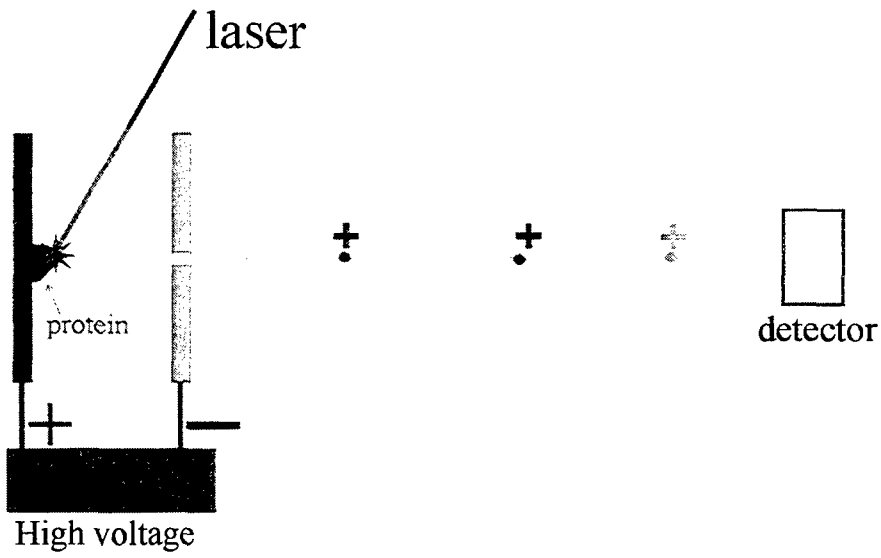

Fig. 5 A schematic illustration of the working principle of a protein chip.

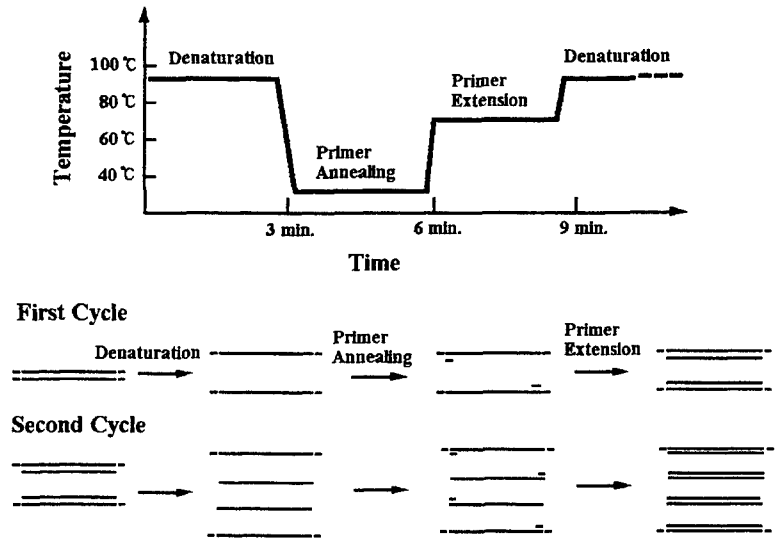

Fig. 6. Thermal cycles in polymerase chain reaction (PCR) for DNA amplification.

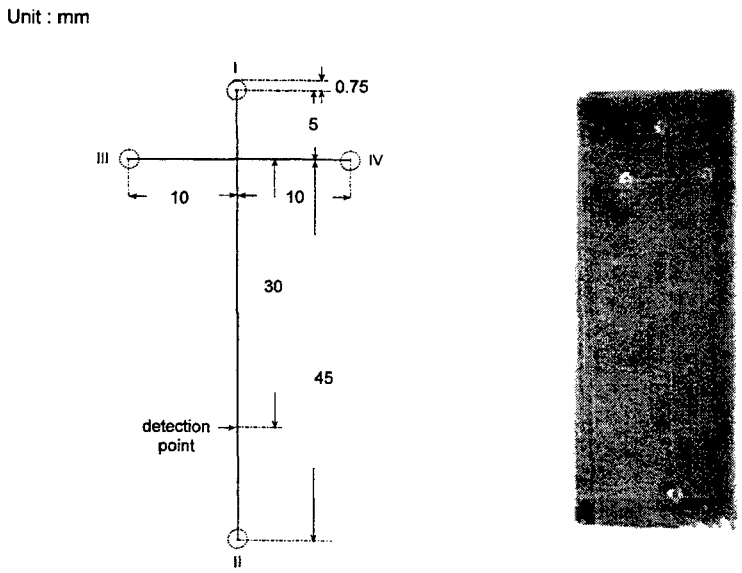

Fig. 7. A schematic illustration of a typical capillary electrophoresis chip fabricated on PMMA by a team at the National Cheng Kung University.[21]

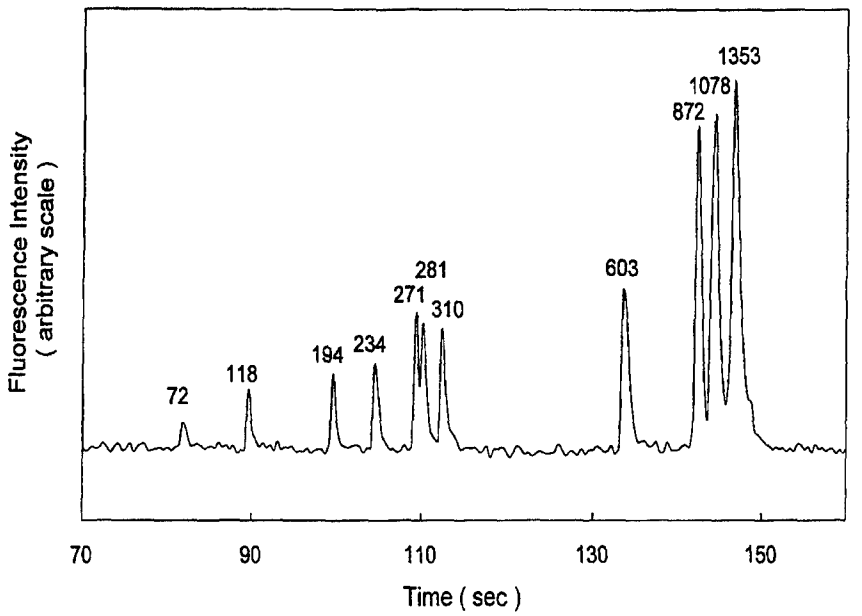

Fig. 8. Electropherogram from a capillary electrophoresis chip shows that all 11 fragments of ϕX174RF DNA digested by the restriction enzyme *Hae*III can be identified in less than 3 minutes.[21]

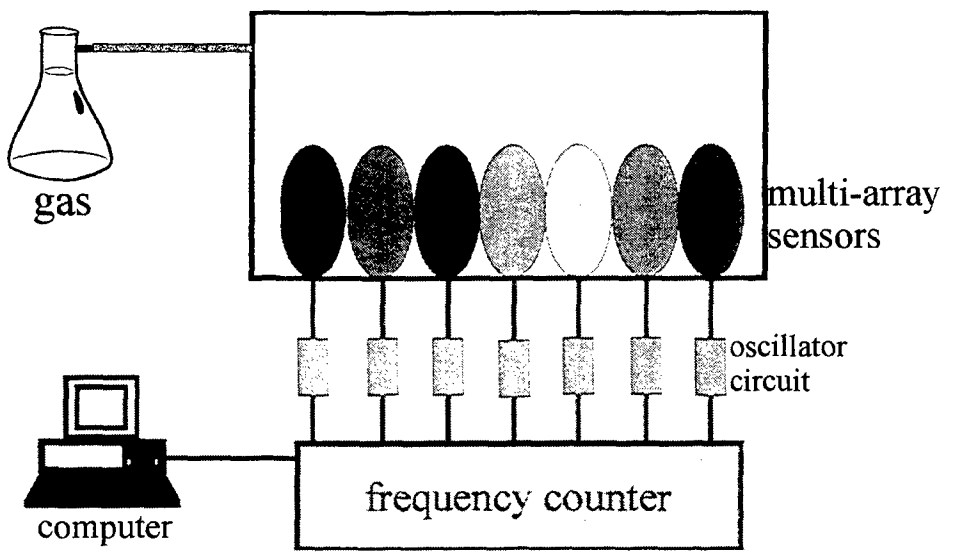

Fig. 9. A schematic illustration of the working principle of an electronic nose.[23]