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Review

Microchip electrophoresis-based separation of DNA

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Miniaturized instruments have developed very quickly in the last decade. This review is focused on the microchip electrophoresis-based separation of DNA. Fundamentals, including the chip format, substrates and fabrication technologies, fluid control, as well as various detection methods, are summarized. Array electrophoresis microchip and the on-chip integration of electrophoresis with other systems are introduced as well. In addition, the application of microchip electrophoresis in DNA sizing, genetic analysis and DNA sequencing are also presented in this paper.

Keywords: Microchip electrophoresis; Microfabrication; Array electrophoresis microchip; Genomics; Review

1. Introduction

Miniaturization of analytical and bioanalytical instruments has developed rapidly in the past 10 years. Up till now, different kinds of micro total analysis systems (μTAS) have been presented, and to different extents, applied into practical analyses. Among them, microchip electrophoresis has been regarded as an emerging new technology that promises to lead the next revolution in chemical analysis [1] because of its prominent advantages, such as high efficiency, high throughput, easy operation, and low consumption of samples and reagents.

Since the first demonstrations in 90's by Manz et al. [2] and Harrison et al. [3], microchip electrophoresis has been keeping on developing. Besides the further research carried out on single-channel microchips [4–10], capillary array electrophoresis (CAE) [11–15] on microplates and the integrated devices with different functionalities [16–20] have also been realized. Up till now, with the increasing interests from different fields, more and more people have devoted into the research on microchip electrophoresis. At present, many important reviews on microchip electrophoresis have been

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published [1,21–32], and special issues have also been printed by *Electrophoresis* in the passed 3 years [33–36]. In addition, some commercial instruments, such as Agilent 2100 Bioanalyzer, Shimadzu MCE 2010, and Hitachi SV 1100 and SV 1210, have come into being, which greatly promotes the further application of microchip electrophoresis. Besides the analyses of peptides, proteins, and chiral compounds, chip-based separation of DNA has been paid great attention, especially in the field of clinical diagnosis. Accordingly, in this review, our focus is put on this aspect, together with the necessary introduction of some basic knowledge of microchip electrophoresis and its recent developments.

2. Fundamentals

2.1. Chip format

Although microchips with stationary phases through inner-wall coating [37], microfabricating support pillars [38], packing particles [39], and in situ polymerization [8] have been developed, most electrophoresis microchips are in the format of open tube filled with suitable buffer. The effective lengths of the microchannel are about several centimeters with width and depth respectively of 10–100 and 15–40 μm.

The design of microchannels on electrophoresis chips has undergone significant development. The first one was fabricated on a planar glass substrate with dimensions of $14.8 \times 3.9 \times 1$ cm^3 [3] (Fig. 1A). Two years later, miniaturized chip with a separation channel of 165 mm within an 8×8 mm^2 area was realized through a serpentine column geometry [37] (Fig. 1B). Instead of single-pass microchips, synchronized cyclic capillary electrophoresis (CE) on microchip was also reported, which permits proper selection of optimized channel lengths by switching voltages between pairs of electrodes [40] (Fig. 1C). With the urgent need of high throughput analysis of DNA, CAE on microplates have also been developed with the channel number increasing from 12 to 384. With the microchip shown in Fig. 1D, the genotyping analyses of 384 samples could be completed in less 7 min with greater than 98% success [41]. In addition, multiple-dimensional systems are also of great interest recently because of their increased peak capacity over one-dimensional separation [42]. It could be forecasted that with the fast development of microfabrication techniques and computer-aided design tools, new formats of microchips might emerge soon to meet the needs of practical applications.

2.2. Substrates and fabrication technologies

Although micromachining is advanced for silicon as a substrate, its semiconductor properties are not compatible with the high voltages of microchip electrophoresis, owing to the breakdown problem [43]. Fintschenko and van den Berg have made a thorough discussion on the advantages and disadvantages of silicon for electroosmotically driven separation techniques [44].

Glass has been regarded as a popular substrate for electrophoresis microchips because of its low cost, good optical properties, well understood surface characteristics, and well developed microfabrication methods adapted from the microelectronics industry. Under most situations, the channel of a microchip is fabricated using standard photolithographic and chemical wet etching techniques, and then a second glass plate is thermally bonded on the top of the etched surface [3,37,40–42]. In addition, new kinds of technologies, such as power blasting to form the channels [45] and UV-bonding at room temperature applicable to dissimilar materials bonding and chip integration [46], have been reported. In addition, quartz has also been chosen as the substrate for the fabrication of microchips although the expense is relatively high compared to glass [47].

Polymers, as an attractive alternative to silicon, glass or quartz for the fabrication of microfluidic systems, are of increasing interest now [48]. The methods developed for fabricating microchannels and reservoirs in polymeric substrates include laser ablation, injection molding, compression molding, casting, and X-ray lithography [49,50]. Taken poly(dimethylsiloxane) (PDMS) as an example, the scheme describing the fabrication of enclosed microscopic channels is shown in Fig. 2 [7]. Besides

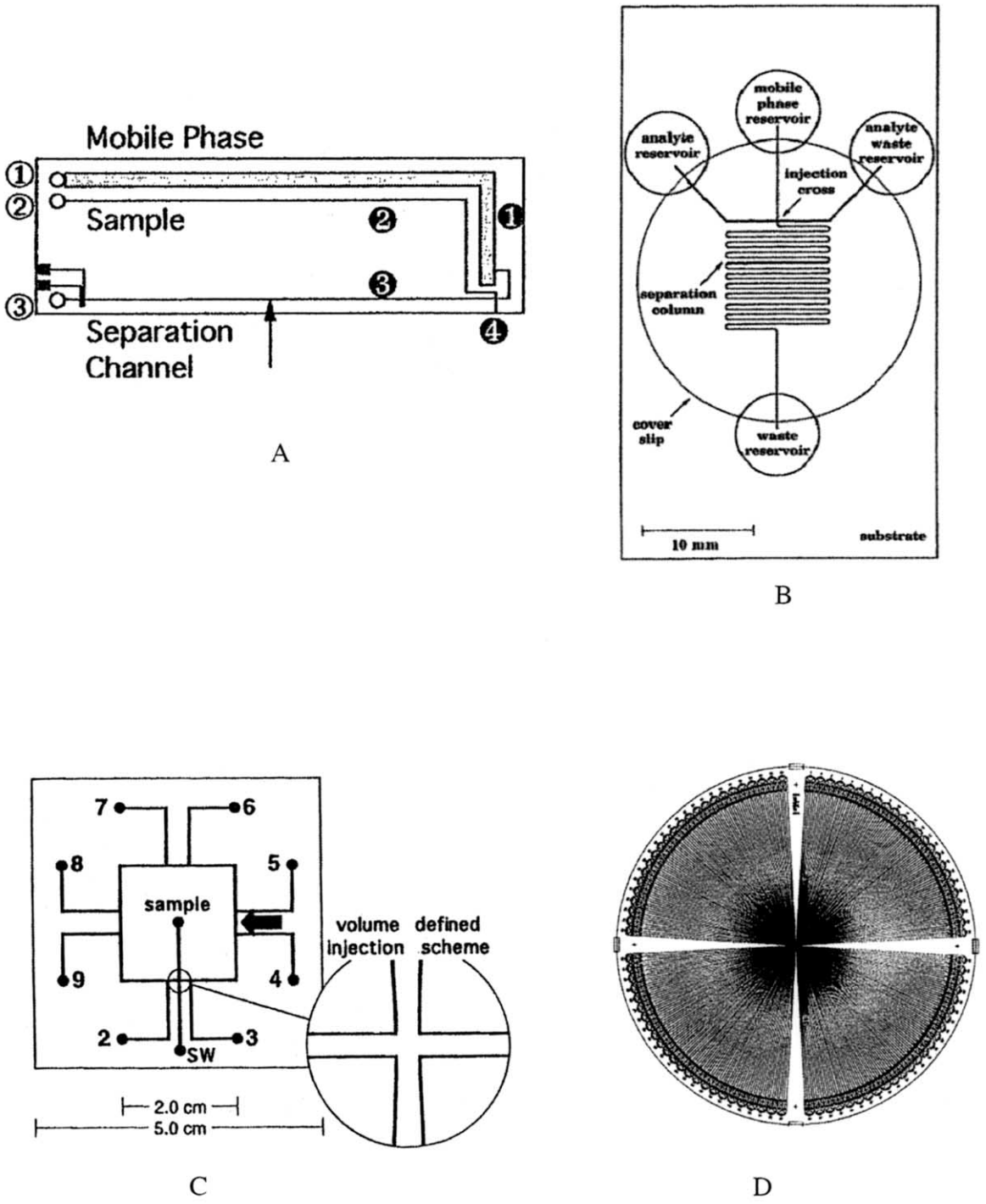


Fig. 1. Various designs of electrophoresis microchips. (A) The first electrophoresis microchip, reprinted with permission from [3], copyright (1992) American Chemical Society. (B) Microchip with serpentine column geometry, reprinted with permission from [37], copyright (1994) American Chemical Society. (C) Cyclic electrophoresis chip with the volume-defined injection scheme, reprinted with permission from [40], copyright (1996) American Chemical Society. (D) 96-channel radial CAE microplate, from [41], with kind permission of Kluwer Academic Publishers.

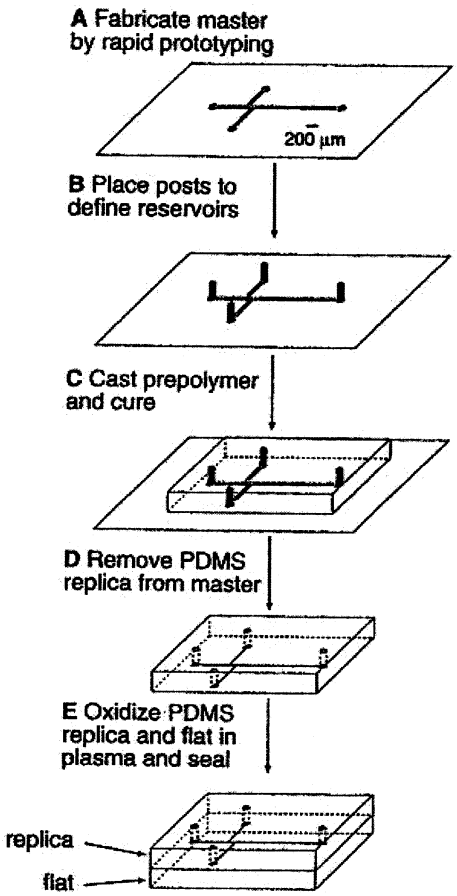


Fig. 2. Scheme describing the fabrication of enclosed microscopic channels in oxidized PDMS. (A) A high-resolution transparency containing the design of the channels, created in a CAD program, was used as the mask in photolithography to produce a positive relief of photoresist on a silicon wafer. The scale bar gives an indication of the thickness and width of photoresist. (B) Glass posts were placed on the wafer to define reservoirs for analytes and buffers. (C) A prepolymer of PDMS was then cast onto the silicon wafer and cured at 65 °C for 1 h. (D) The polymer replica of the master containing a negative relief of channels was peeled away from the silicon wafer, and the glass posts were removed. (E) The PDMS replica and a flat slab of PDMS were oxidized in a plasma discharge for 1 min. Plasma oxidation had two effects. First, when two oxidized PDMS surfaces were brought into conformal contact, an irreversible seal formed between them. This seal defined the channels as four walls of oxidized PDMS. Second, silanol (SiOH) groups introduced onto the surface of the polymer ionize in neutral or basic aqueous solutions and support EOF in the channels. Reprinted with permission from [7], copyright (1998) American Chemical Society.

PDMS, which has been regarded as an extremely promising material for the fabrication of microfluidic systems [51], other polymers, such as poly(methylmethacrylate) (PMMA) [52], polyacrylate [53], and polycarbonate [54] have been successfully utilized as the substrates for electrophoresis microchips.

2.3. Fluid control

In microchip electrophoresis, the fluids are controlled by the electric field. For the sample loading procedure, generally small pinching voltages are applied to the buffer reservoirs to avoid the leakage of samples into the separation channel. After the injector is filled, the separation is initiated by applying an electric field in the separation channel. Both the injector geometry and the electric field distribution are of paramount importance to find the right tradeoff between separation efficiency and detection sensitivity [55–57].

Besides the normal injection, via either a single T or a twin T injector, different methods for on-line sample concentration have been proposed to improve the detection sensitivity. Ramsey et al. incorporated a porous membrane in the microchannel, which allowed the passage of current to establish an electric connection between the separated channels but prevented large molecules, such as DNA, from traversing the membrane [58]. In addition, sample stacking [59,60], micellar sweeping [61] and on-chip solid phase extraction (SPE) [39,62] have also been adopted and the detection sensitivity could be improved up to 3 orders of magnitude.

As far as the separation is concerned, many parameters, such as the column geometry [63–65], sieving matrix [66], separation temperature, and electric field strength [9], might have great effects on the analysis speed as well as the resolution. In addition, the effect of Joule heat should also be taken into account [67]. All these conditions should be considered simultaneously in order to obtain the optimal separation.

2.4. Detection

Sensitive detection is essential in microchip electrophoresis due to the extremely small size of the detection cell. Laser-induced fluorescence (LIF) detection is the most popular detector because of its extremely high sensitivity and compatibility to microchips [3,40–42,47,53–63]. However, the most obvious disadvantage of LIF detector is that derivatization with a fluorophore is necessary. Although UV detector can avoid this problem, the path length-dependence seriously limits its sensitivity [47,68]. Another attractive detector is to interface the output of a microchip system with a mass spectrometry, which is capable of generating very detailed information on a sample in a short time. Furthermore, very small samples required are well matched to the flow rates of microchip systems [2,69–71]. Electrochemical detection has also developed very quickly and Lunte et al. published a good review on this aspect [72]. Chemiluminescence detection [73], normal Raman spectroscopy [74], and a universal detection system based on refractive index measurements [75] have been developed as well.

3. CAE on microchips

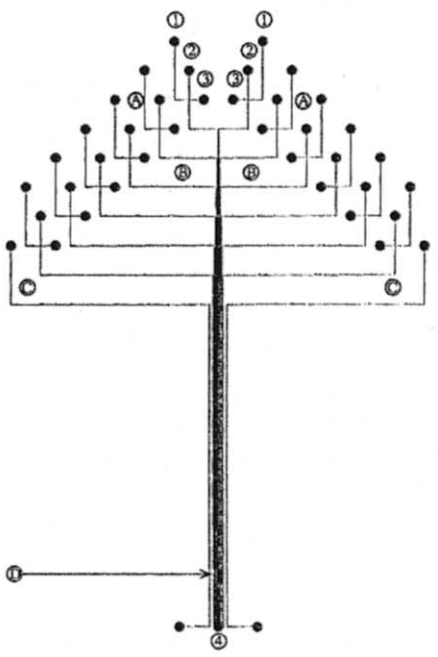
Although single-channel microchips have enabled high-speed separation, to realize high throughput analysis, parallel analyses with multiple-channel on one microchip are indispensable. Accordingly, in the passed few years, CAE on microchips has developed very quickly on both chip design and the corresponding detection systems. Since some reviews on this aspect have been published recently [76,77], in this paper, we would just make a brief summary with the newest papers included.

An electrophoresis microchip with 8 independent microchannels has been designed by Landers et al. and laser beam scanning driven by an acoustic-optical deflector is presented for the multichannel LIF detection [13,78]. By using a laser-excited galvo-scanner, four-color sequencing in 16 parallel channels has also been demonstrated on microchips by Jovanovich et al. [14]. In addition, Went et al. have fabricated 48 parallel channels on a piece of Borofloat glass with 165 mm length and 100 mm width. The system uses a dual laser excitation source and a highly sensitive charge-coupled device detector allowing for simultaneous detection of many fluorescence dyes [79]. It should be pointed out that the group of Mathies has made great contribution to the development of array electrophoresis microchips. They have successfully developed 12-channel [11] (Fig. 3A), 48-channel [12] (Fig. 3B), and 96-channel (Fig. 3C) [15], and 384-channel chips (Fig. 1D) [41], which could greatly increase the analysis rate over current commercial CAE technologies.

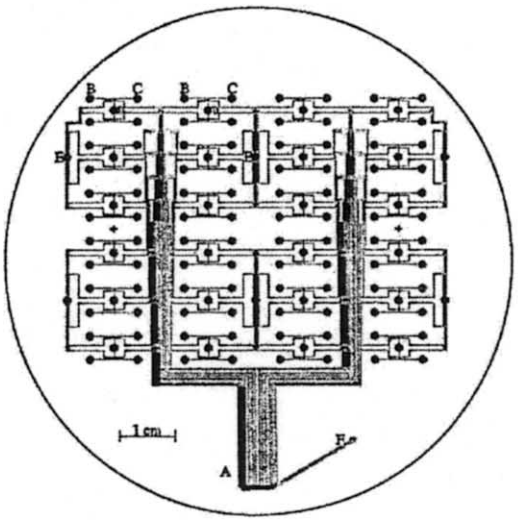
4. Integrated devices

Besides the high throughput analyses by multiple-channel microchip electrophoresis, the integration of different processes on a chip is of great significance. In this paper, the microchip electrophoresis based integrated devices for DNA analyses are presented.

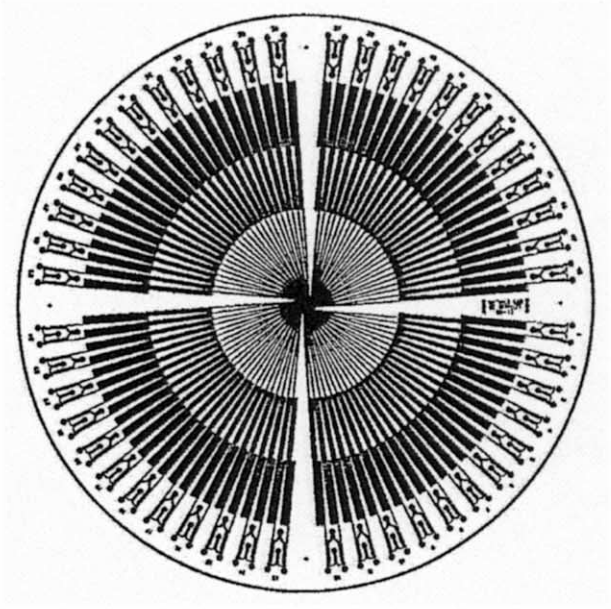
Ramsey et al. first realized the integration of the digestion of plasmid pBR322 by the enzyme *Hin*fI and the consequent fragment analysis by electrophoresis within 5 min [16]. In the newly published paper, Burns et al. have developed the concept of heat integration, which has shown potential in the construction of multiple on-chip reaction systems and the further integration with microchip electrophoresis [80]. A functional integration of polymerase chain reaction (PCR) and CE on a microchip was initially developed by Northrup et al. in 1996 [17]. Later on, the steps of cell lysis, multiplex PCR amplification, and electrophoretic analysis executed sequentially on a monolithic microchip device have also been performed by the group of Ramsey [18]. Burns et al. [19] and Mathies et al.'s [20] work on the integration of PCR and electrophoresis on microchip could be regarded as the masterpieces of integrated miniaturized devices. In the former's work, they successfully combined a nanoliter liquid injector, a sample mixing and positioning system, a temperature-controlled reaction chamber, an electrophoretic separation system and a fluorescence detector on a microchip.



A



B



C

Fig. 3

While in the latter's work, an exquisite design on the fluidic control systems made stochastic PCR amplification of single DNA template molecules following by electrophoresis analysis on an integrated microchip possible. Besides the traditional glass and silicon-based microchips, the integration of PCR and CAE on PMMA substrates has also been developed [81]. More recently, Harrison et al. have realized the integrated microfluidic electrophoresis system for the analysis of genetic materials using signal amplification methods [82]. In addition, Landers et al. have shown promising results on incorporating silica-based SPE system into a microchip platform, which might find utility in a variety of genetic analysis protocols [83].

From the above-mentioned work, we could foresee that the availability of portable and reliable integrated devices might facilitate the further application of microchips.

5. Applications

A development of applications for a new technology is always a confirmation that the technology is maturing. The application of microchip electrophoresis in DNA analysis has undergone a fast development in the following aspects.

5.1. DNA sizing

The first DNA separations in a microchip were reported by Effenhauser et al. in 1994 [84]. A mixture of DNA oligomers with length from 10 to 15 bases was separated with high efficiency. Later on, many fundamental studies of microchip electrophoresis have been carried out with DNA ladders as examples [9,85]. Ueda et al. have proposed the field-inversion electrophoresis on a microchip device and demonstrated its potentials in the fast separation of short DNA fragments [86]. DNA digestion fragments have also been analyzed by microchip electrophoresis, either with a single-channel [87] or multiple-channels [88]. Besides the reduction of analysis time, high resolution obtained within channels with limited length is also very important. Baba et al. have recently proposed the stepwise gradient of linear polymer concentration in the microchannel. With different pore sizes coexisted in the sieving matrix, obvious improvements on the resolution of DNA fragments have been obtained [10].

5.2. Genetic analysis

The sequencing of human genome has been almost completed in early 2001. Human Genome Project (HGP) will quickly move on to the post genome sequencing area, including the analyses of functional genomics, single nucleotide polymorphism (SNP), and gene mutation [89]. Microchip electrophoresis with the necessary integrated processes has played an important role in genetic analysis. By far, several reviews on this aspect have been delivered [90–92].

Fast analyses of the candidate gene for hereditary hemochromatosis [11,12], alleles of the D1S80 locus [93], multiplexed short tandem repeats [94], and specific PCR products of Hepatitis C virus [95] have been published. Compared to the traditional methods by capillary gel electrophoresis and CE, the analysis efficiency of microchip electrophoresis could be improved by 10–100 ×, which is quite meaningful for the clinical diagnosis. Rapid detection of deletion, insertion and substitution mutations by combining allele-specific DNA amplification with heteroduplex analysis by microchip electrophoresis has been accomplished within 130 s [96,97]. By array electrophoresis microchips, the analysis speed could be further improved. With multi-channel microchips, 96 allele-specific amplification products related to *HFE* gene could be analyzed within 100 s [98,99]. In addition, fast detections of SNP and gene mutation by enzy-

Fig. 3. Configurations of array electrophoresis microchips. (A) 12-channel for 12 samples, reprinted with permission from [11], copyright (1997) American Chemical Society. (B) 8-channel for 96 samples, reprinted with permission from [12], copyright (1998) National Academy of Sciences, USA. (C) 96-channel for 96 samples, reprinted with permission from [15], copyright (2002) National Academy of Sciences, USA.

matic mutation detection [100], single-strand conformation polymorphism analysis [101], and restriction fragment length polymorphism analysis [102] with the combination of microchip electrophoresis have also been reported.

5.3. DNA sequencing

CAE has contributed greatly to HGP since it has fastened the DNA sequencing. However, improvements on technology are still needed to map the genomes of individuals and other species. Accordingly, the instruments for the next generation might rely on microchip.

DNA sequencing on a microchip was first demonstrated in 1995 by Woolley and Mathies [6]. At that time, single-base resolution to approximately 150 bases was achieved in 540 s with 97% accuracy. Under the stimulation of HGP, DNA sequencing on microchips has experienced a fast improvement [28,103–109]. Not only multiple-channel sequencing has been realized [14,15], but a specific sequencing matrix has been proposed as well [108]. In Mathies's recent work, DNA sequencing data from 95 successful lanes out of 96 lanes run in parallel were batch-processed, producing an average read length of 430 bp at a rate of 1.7 kb/min [15]. This system permits lower reagent volumes and lower sample concentrations, and it also presents numerous possibilities for integrated sample preparation and handing.

6. Prospect

Microchip-based technology has been forecasted to be fundamental to the post-genome sequencing era, and will be applicable to the analysis of DNA, mRNA, protein, and metabolites. Recent progresses in microfabrication and nanofabrication technologies based on computer-chip technology have introduced a new research area for integrated microchip and nanochip technology [110]. In the near future, so-called exa-bioinformatics, which integrates both ultra-fast information technology and exa-sequencing technology based on microchip and nanochip, will be launched and enable us to obtain the huge amounts of information required for personalized medicine.

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