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Journal of Chromatography A, 924 (2001) 265–270

JOURNAL OF CHROMATOGRAPHY A

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Microfabricated capillary array electrophoresis DNA analysis systems

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Abstract

Microfabricated ''laboratory-on-a-chip'' systems are revolutionizing all aspects of genetic analysis. The development of capillary array electrophoresis (CAE) microchannel plate devices makes possible the performance of 96 or more high-speed separations in parallel on a single wafer-scale device. The fluorescently labeled DNA samples are detected within the microchannels with a novel four-color rotary confocal fluorescence scanner. The capabilities of this system for genotyping are demonstrated through multiplex separations of short tandem repeat and hereditary haemochromatosis allele-specific amplicons. Furthermore, with newly developed folded channel designs that maintain high resolution, these CAE microplate systems are used to perform 96 high-quality DNA sequencing separations in parallel to ~500 bases per capillary in less than 30 min. These densely packed microfabricated device technologies will facilitate the even more rapid collection of vast amounts of genetic data in the future.

Keywords: Chip technology; Capillary array electrophoresis; Instrumentation; DNA

1. Introduction

Microfabricated capillary electrophoresis devices offer numerous advantages for genetic analysis including reduced sample volumes, higher speed and sensitivity, and the ability to pack separation channels densely into smaller monolithic platforms [1–3]. The use of a radial channel array format simplifies the design, fabrication and function of capillary array electrophoresis (CAE) microplates and allows for even greater throughput on these devices [4]. These radial CAE microplates were recently used with a novel rotary scanning confocal fluorescence detector to analyze 96 genotyping samples in parallel in $<$100 s [4]. Energy transfer (ET) labeled allele specific amplicons and controls, generated from the 96 samples, were separated in 3% denaturing polyacrylamide matrix with single base-pair resolution in under 10 min [5]. Additionally, microfabricated CE channels only 7-cm long have been used to produce $>$500 base-pair (bp) four-color DNA sequencing reads in under 30 min [6,7].

To permit the layout of high-density microchannel devices and to facilitate the performance of high resolution separations in parallel, designs encompassing the folding of microchannels have been explored [8]. Folded channel designs called ''hyperturns'', in which the microchannel is tapered before the turn and widened after the turn, were shown to function at 95% the efficiency of a straight channel of comparable length [8]. Our goal here is to

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00852-4

demonstrate the full capabilities of these radial CAE devices through multiplex genotyping assays including short tandem repeat (STR) amplicon analysis and hereditary haemochromatosis (HHC) diagnosis. Additionally, these chips have the ability to gather large amounts of sequencing data in parallel through high-speed separations on CAE chips that utilize hyper-turn geometries.

2. Materials and methods

CAE microplates were fabricated at the University of California at Berkeley Microfabrication Laboratory as described previously [4,9]. Microplate designs are presented in Fig. 1. The straight channel design is optimum for rapid high-performance genotyping analysis (panel A) while the hyper-turn channel design (panel B) is more suited to long read DNA sequencing. Isotropic etching of the 150 mm diameter substrate with HF formed ~110 μm wide by 50 μm deep channels for the straight channel design. The folded channel design utilizes ~200 μm wide by 30 μm deep channels which narrow to 65 μm in the hyper-turns. The distance along the separation capillary from the 250 μm twin-T injector to the detection point is 55 mm for the straight channel design (panel C) while the effective separation length for the folded channel design is extended to 160 mm (panel D). The design and function of the rotary scanning confocal fluorescence detector is discussed extensively in Shi et al. [4]. A schematic of the scanner is presented in Fig. 1E.

3. Results

3.1. High-performance genotyping

The straight channel CAE microplate has been utilized for numerous multiplex high-performance genotyping assays. These include the simultaneous analysis of three single nucleotide polymorphism (SNP) alterations associated with hereditary haemochromatosis in a population of 96 samples [5]. To explore the ability of the CAE microplates to perform higher order multiplex STR analysis, five different amplicons were mixed with a sizing ladder and separated in under 8 min (Fig. 2). These amplicons were fluorescently labeled with a recently developed ET cassette technology [10]. The samples labeled with three different ET cassettes were separated against a DNA sizing ladder labeled in a fourth color. Data were processed as described by Medintz et al. [5]. The amount of amplicon used for each separation (0.5 μl) represents 1/100th of the total amount of amplicon generated. The analysis time, <8 min, represents a 10-fold improvement over the analysis time required using commercial capillary array electrophoresis technology.

The separation presented in Fig. 2B shows the genotyping of a sample at three HHC associated loci, C282Y (845g→a), H63D (187c→g), and S65C (193a→t) [5]. Allele specific amplifications (ASAs) generated both wild-type-specific (red) and mutant-specific (blue) amplicons along with polymerase chain reaction (PCR) controls, also in red. Note the single bp resolution achieved between the 280 bp standard and the 281 bp PCR control. This analysis was completed in <10 min on the CAE chip. This type of analysis demonstrates the rapid, multiplex capabilities of CAE based genotyping separations.

CAE microplate technology is particularly well suited to genotyping assays requiring the rapid separation of large numbers of multicolor amplicons. Studies such as linkage analysis, loss of heterozygosity testing, and gene mapping fall into this category of genetic assay as well as forensic identity typing. These studies require the screening of thousands of individuals across large numbers of microsatellite loci using STR site-specific amplicons. The full implementation of microfabricated CAE based genotyping assays will allow for an almost decadic leap over the capabilities of current technology.

3.2. High speed DNA sequencing

The ability to rapidly collect large amounts of DNA sequencing data is also a major goal of our ''laboratory-on-a-chip'' genetic analysis project. However, the DNA sequencing data must also be of very high quality. The implementation of the hyper-turn geometry for CAE microchannel design represents a major step forward. This technology increases separation channel length and feature density

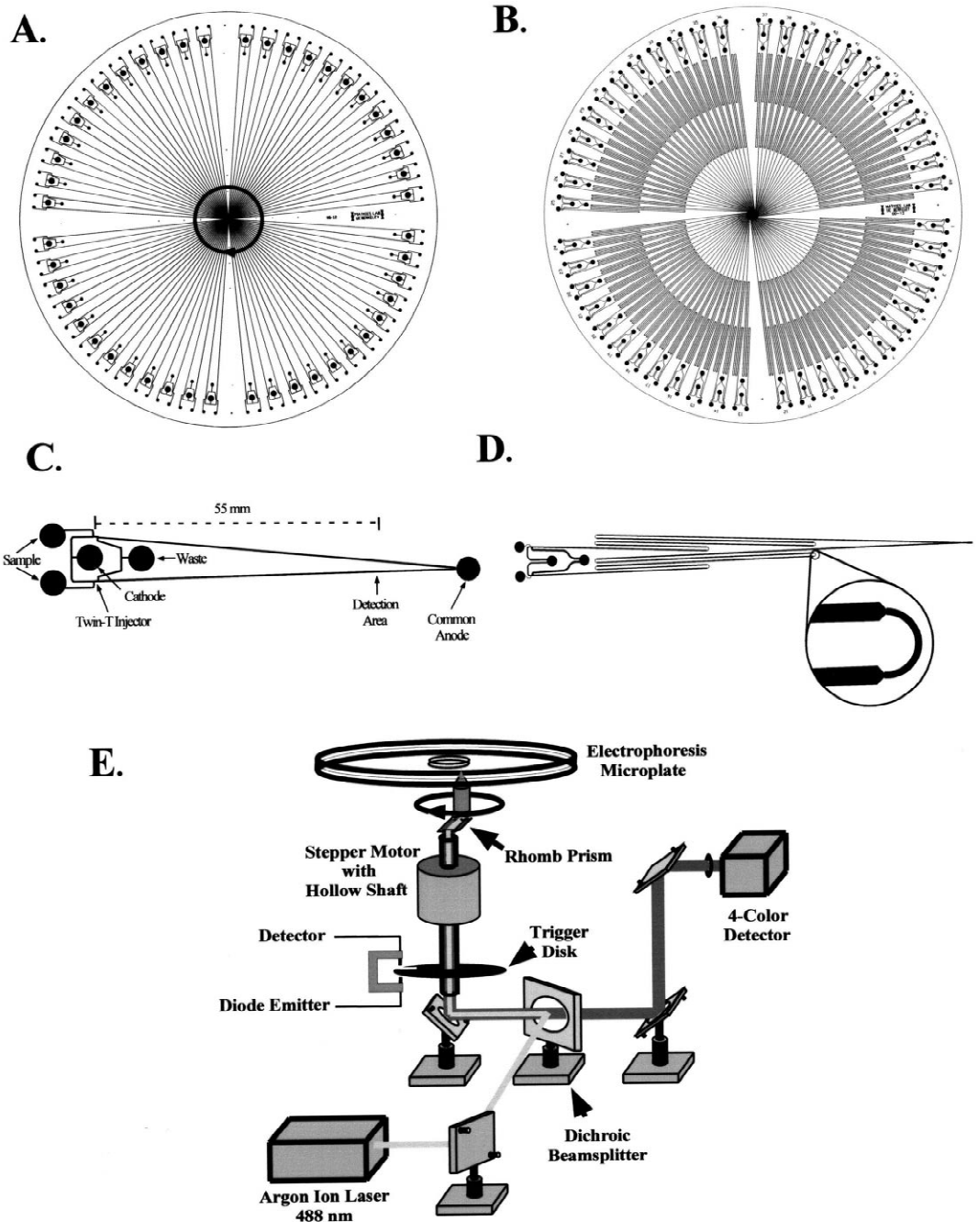

Fig. 1. Design of the 96-channel CAE microplate and radial scanner. (A) Mask pattern used to form the 96 straight channel radial CAE microplate on a 150-mm diameter wafer. The black circle in the center of the plate indicates where the laser beam from the rotary scanner interrogates the separations. (B) Mask pattern used to form a 96-channel folded turn radial CAE microplate. Each microchannel has an effective separation length of 160 mm. (C) Enlarged view of a set of individual straight channel capillaries showing the common cathode, waste and anode reservoirs shared between the two capillaries. (D) Enlarged view of the hyper-turn geometry and a set of individual folded turn capillaries (E) Schematic of the radial confocal microplate scanner. Adapted from Shi et al. [4] and Medintz et al. [5].

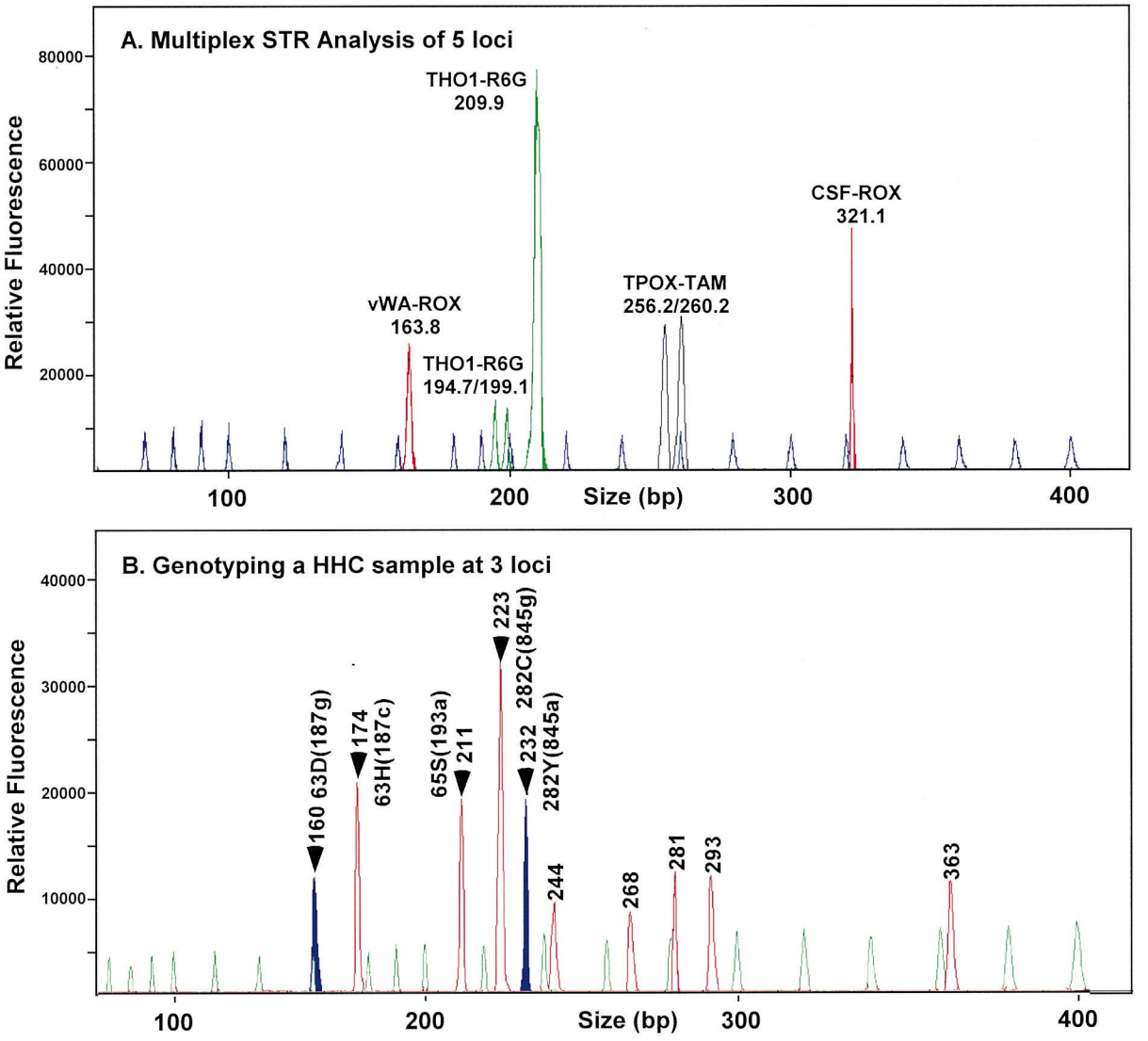

Fig. 2. (A) Four-color electropherogram demonstrating multiplex STR analysis. The vWA-ROX (6-carboxy-X-rhodamine, red), and THO1-R6G (6-carboxyrhodamine-6G, green) amplicons were generated from K562 cell-line DNA. The THO1-R6G, TPOX-TAM (carboxytetramethylrhodamine, black) and CSF-ROX amplicons were generated from CEPH donor DNA. The amplicons were mixed together and sized against the FAM (fluorescein) Mapmarker Sizing Standard (blue). The standard consist of 20 fragments (70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380 and 400 bp). (B) CAE microplate multiplex SNP genotyping analysis of three HHC associated loci. Using allele-specific (AS) amplification, wild-type ET-ROX (red) labeled AS amplicons are generated with sizes of 174 bp (63H-187c), 211 bp (65S-193a) and 223 bp (282C-845g). The other ET-ROX (red) peaks found at 244, 268, 281, 293 and 363 bp are PCR controls. The 20 TET-labeled (tetrachlorofluorescein) sizing standards are detected in the green channel. Note the presence of the extra ET-R110 (blue) peaks at 160 and 232 bp, generated by the presence of the 63D-187g and 282Y-845a mutant alleles, respectively.

without compromising performance while maintaining a small chip size [8].

Previous work has demonstrated that read lengths of >500 bp are feasible with a 7-cm long separation channels in under 30 min [6,7]. Fig. 3 presents the data processed from a single channel separation of

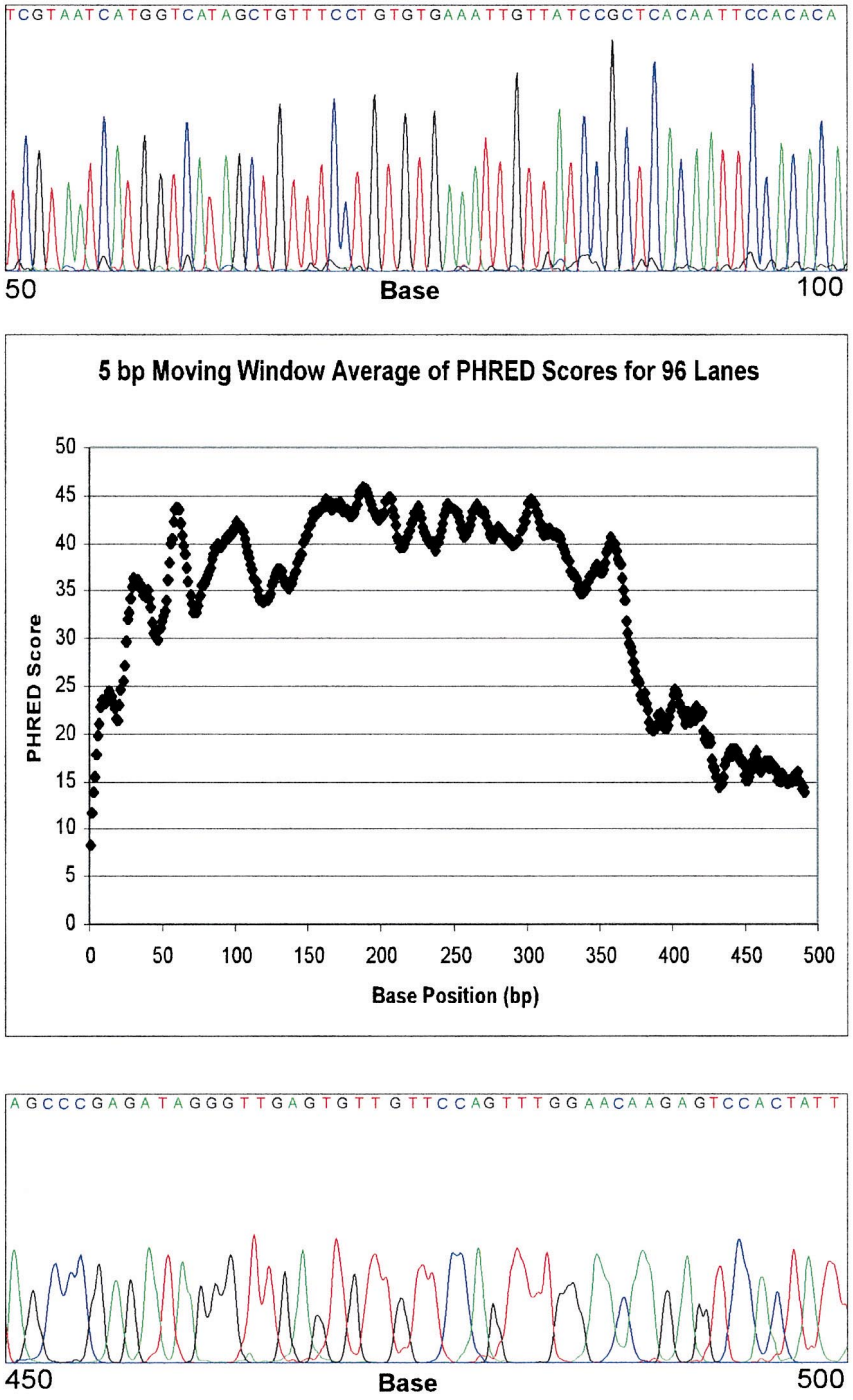

Fig. 3. Four-color DNA sequencing traces from a single 16-cm long microchannel on the folded turn radial CAE microplate. Four-color M13 standard DNA sequencing extensions were separated in <30 min and the resulting electropherogram collected and processed. The resulting data from 50 to 100 bases and 450 to 500 bases is displayed along with a plot of overall PHRED score for a 96-channel microchannel separation.

four-color M13 Standard DNA sequencing extensions on a 16-cm long hyper-turn CAE chip in <30 min. The processed base calls from 50 to 100 bp and 450 to 500 bp are presented along with the overall PHRED score for a 96-channel microchip separation. The PHRED program is used to assign quality values to each base call [11]. A value of 20 indicates a predicted error rate of <1% while a value of 30 indicates an error probability of only 1/1000. These values provide the best objective measure of separation quality and sequence accuracy [11]. As is seen in Fig. 3, an overall PHRED quality score above 20 is achieved for ~80% of the >600 bp sequence length. The PHRED plot also indicates that no bases were ''un-called'' due to poor separation quality. These DNA sequencing results demonstrate the capabilities of CAE microplates with hyper-turn channels for both high-performance separations and rapid analysis. Results on the successful use of these chips for the parallel sequencing analysis of 96 samples are in progress.

4. Discussion

CAE microchannel plate designs are being optimized for maximum performance for sequencing and genotyping. This includes arraying 96 channels in a radial format to achieve high throughput and utilizing high-resolution turn designs to extend separation efficiencies. Straight channel designs are utilized for high-performance genotyping separations while the hyper-turn chips are more suited to DNA sequencing where long separation lengths are required. The capabilities of these CAE chips are being explored by performing multiplex genotyping assays and 96 parallel DNA sequencing analyses. When fully implemented, this technology will provide an almost decadic leap over the capabilities of conventional commercial CAE instruments employing discrete capillaries.

Acknowledgements

The authors thank Jim Scherer for invaluable aid in the construction and operation of the microplate scanner. I.L.M. is supported by NIH Program Project grant P01 CA77664 which is a collaboration with Johns Hopkins University. This research was also supported by NIH grant HG01399 and by the Director, Office of Science, Office of Biological and Environmental Research of the US Department of Energy under contract DE FG91ER61125.

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