DE-FG03-91ER61125
High Performance Labeling, Separation and Detection Methods
for Genome Analysis
Final Report
Richard A. Mathies, PI

1. Advanced Integrated PCR reactors

In recent work, Eric Lagally has focused on the complete integration of the various heaters and temperature sensors with the goal of making a fully integrated portable PCR-CE device. Toward this end, thin film heaters have been fabricated on the outside of the wafer and a four-wire resistance temperature detector (RTD) has been fabricated *within the PCR reactor chamber itself*. The reduced thermal mass, faster thermal response time and the intimate and direct temperature monitoring of the reaction produce a PCR reactor with 20 C/s response times. This system has been used to perform a complete buccal swab genetic determination in a one-step process in 20 min. In addition, we have shown that infectious pathogens such as enterohemorrhagic *E. coli* and antibiotic resistant *Staphylococcus* can be detected and genotyped on this portable apparatus by PCR amplification followed by CE analysis of the products (7, 11). Eric has now completed his thesis and will be taking up a postdoctoral position.

2. Integrated microvalves

Our previous valve fabrication methods require the manual placement of latex or hydrophobic membrane pieces in drilled valve seats. To solve this problem, Will Grover has been working on new microfabrication methods utilizing wafer sized thin sheets of PDMS as the active valve material. With this multilayer approach, valves are fabricated where desired by drilling two adjacent holes through the valve wafer. The entire surface of this wafer is then covered with a thin PDMS membrane and actuation of the membrane is provided by contacting the PDMS layer with a plastic or glass pneumatic manifold layer. In this way we have fabricated dense arrays of 144 valves as well as vents, reactors and pumps in a single trivial step on a 4" wafer with 100% yield (2, 4, 5). This technology now permits the fabrication of arrays of PCR or thermal cycling reactors coupled to purification chambers and CE systems on a single substrate. This work is also the subject of a pending patent application. Will is now working on non-DOE related projects.

3. Integrated optical detectors

Dr. Kamei has developed, in collaboration with Xerox, an integrated amorphous Si:H photodiode detector and demonstrated its utility for detecting simple PCR fragment separations on chips. This detector is the subject of a pending patent application. This

work is described in the MicroTAS 2002 Proceedings (6) and in a more complete paper recently published in *Analytical Chemistry* (10). The hybrid integrated detection system consists of a half-ball lens, a dielectric interference filter with a pinhole, and an annular a-Si:H photodiode fabricated on a glass substrate. Using this integrated detection system, the limits of detection for fluorescein is 17 nM, and DNA fragment sizing and chiral analysis of glutamic acid are successfully performed on-chip. Dr. Kamei has now returned to Japan.

4. Integrated genetic microprocessors

In recent work, Brian Paegel has developed a revolutionary new method for integrated nanoliter DNA sample clean-up and purification for DNA sequencing that more fully exploits the low-volume sample preparation capabilities of our microsystems (3, 9). In this method a methacrylamide monomer is labeled with a capture oligonucleotide having a sequence that is complementary to the vector just 3' of the universal sequencing primer. The sequence is chosen so that it will capture only extended DNA sequencing ladder components optimally at a temperature of ~ 50 C. This oligo-labeled monomer is then polymerized to form a viscous linear acrylamide gel that can be easily pumped into a 100 nL capture chamber integrated on our sequencing chip. A few microliters of raw thermal cycling product is then placed in a reservoir on-chip and electrophoresed through the stationary capture matrix and only the extension products are bound. The high mobility salts, NTP's etc are swept through the reactor and the low mobility template does not enter the capture gel. *In this way we can concentrate the 3 microliter of reaction mixture into 10-20 nL volume in 120 s effecting a 200-fold concentration and quantitative product capture while also desalting the sample.* Heating the chip to the 60 C sequencing temperature releases the products for injection and analysis. We have demonstrated over 600 bp reads by using these methods. This work coupled with the valves described above establishes the base technologies needed to develop a complete sequencing lab on a chip as described in our recent *Current Opinions in Biotechnology* article (9). Brian has now filed his thesis and will take up a postdoctoral position at UCSD.

Publications

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3. Paegel, B. M., Yeung, S. H. I. and Mathies, R. A. Microchip Bioprocessor for Integrated Nanovolume Sample Purification and DNA Sequencing, *Anal. Chem.* **74** , 5092-5098 (2002).
4. Grover, W. H., Skelley, A. M., Liu, C. N., Lagally, E. T., and Mathies, R. A. Monolithic Membrane Valves and Diaphragm Pumps for Practical Large-Scale Integration into Microfluidic Devices, *Sensors & Actuators B*, **89**, 315-323 (2003).
5. Grover, W. H., Skelley, A. M., Liu, C. N., Lagally, E. T., and Mathies, R. A. Practical Valves and Pumps for Large-Scale Integration in Microfluidic Devices, *Micro Total Analysis Systems 2002*, Volume 1, eds. Y. Baba, S. Shoji and A. van den Berg, Kluwer Academic (Dordrecht, The Netherlands), pp 136-138.
6. Kamei, T., Scherer, J. R., Paegel, B.M., Skelley, A. M., Street, R. A., and Mathies, R. A. Integrated Amorphous Silicon Photodiode Detector for Microfabricated Capillary Electrophoresis Devices, *Micro Total Analysis Systems 2002*, Volume 1, eds. Y. Baba, S. Shoji and A. van den Berg, Kluwer Academic (Dordrecht, The Netherlands), pp 257-259.
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8. Blazej, R., Paegel, B. M., Emrich, C. A., and Mathies, R. A. Microfabricated capillary array electrophoresis: implementation and applications, In *Lab-on-a-Chip: Miniaturized Systems for (Bio) Chemical Analysis and Synthesis*, ed. by A. van den Berg and R. E. Oosterbroek, (Elsevier Science, Amsterdam), pp. 231-248 (2003).
9. Paegel, B. M., Blazej, R. G., and Mathies, R. A. Microfluidic Devices for DNA Sequencing Sample Preparation and Electrophoresis Analysis, *Current Opinions in Biotechnology*, **14**, 42-50 (2003).
10. Kamei, T., Paegel, B. M., Scherer, J. R., Skelley, A. M., Street, R. A. and Mathies, R. A. Integrated Hydrogenated Amorphous Si Photodiode Detector for Microfluidic Bioanalytical Devices, *Anal. Chem.*, **75**, 5300-5305 (2003).
11. Lagally, E. T., Scherer, J. R., Blazej, R. G. and Mathies, R. A. Genetic Analysis Using a Portable PCR-CE Microsystem, in *Micro Total Analysis Systems 2003*, Vol, 2, eds. M. A. Northrup, K. F. Jensen and D. J. Harrison, pp. 1283-1286.