AUTOMATED MICROFLUIDICS FOR GENOMICS

Deirdre R. Meldrum[1], *Senior Member, IEEE,* William H. Pence[2], Stephen E. Moody[2], David L. Cunningham[2], Mark Holl[1], Peter J. Wiktor[3]

[1]Department of Electrical Engineering, University of Washington, Box 352500, Seattle, WA 98195-2500; [2]Orca Photonic Systems, Inc., 14662 NE 95th St., Redmond, WA 98052;
[3]Engineering Arts, 7236 91st Ave. SE, Mercer Island, WA 98040

Abstract--**The Genomation Laboratory at the University of Washington is developing an automated fluid handling system called "Acapella" to prepare microliter reactions for genome analysis. The system prepares 5,000 samples in 8 hours for general-purpose chemistry analysis including DNA sequencing reaction preparation.**
***Keywords*--Automation, DNA sequencing, microfluidics, biotechnology, biomechatronics, genomics**

I. INTRODUCTION

Motivated by the Human Genome Project (HGP) and the biotechnology revolution, an exponential growth in genome automation has occurred over the past several years [1]. Solutions include robots that mimic manual procedures in the laboratory, automated systems that improve performance for specific tasks, to microfabricated chips that perform microfluidic analysis of DNA samples. For a review of genome automation, see [2][3]. For an introduction to DNA sequencing and the automation of it, see [4].

In the Genomation Laboratory at the Univ. of Washington (http://rcs.ee.washington.edu/GNL/genomation.html) and with Orca Photonic Systems, Inc. (Redmond, WA), an automated submicroliter fluid sample preparation system called ACAPELLA is being developed. Reactions such as restriction enzyme digests, polymerase chain reactions (PCRs), and sequencing reactions are prepared in glass capillaries, one per sample, with an automated system that can process 5,000 samples in 8 hours. On-going development includes new, fully automated modules for thermal processing of capillaries, real-time DNA quantitation, and purification of DNA inside of capillaries to prepare the samples for DNA sequencing. Applications of the technology include minimal residual disease quantification and sample preparation for DNA. Preliminary work on the ACAPELLA is presented in [4][5]. This paper presents the current development on the ACAPELLA core processor and the thermal cycling module.

II. SIGNIFICANCE

The goals of the ACAPELLA system are 1) to develop a very high-throughput (5000 samples in 8 hours) fluid handling system with minimal recurring labor costs, 2) to reduce by tenfold the typical DNA sample volumes for Polymerase Chain Reaction (PCR) and sequencing reactions over current practice, with a proportionate reduction in reagent costs, and 3) to develop a closed sample-processing pipeline. The core technology comprises a system capable of mixing an incoming DNA sample with appropriate reagents in a capillary under full automation.

In [5] the significance of the throughput, cost reduction, process benefits, and cost/benefit analysis of the ACAPELLA system is described. In short, the system prepares small 0.5 - 2 µl reaction volumes, maintains a 100 to 300 picoliter reagent dispense resolution, retains high mixing precision and quality in small volumes, and demonstrates the ability to achieve high quality, reproducible biology without contamination. The high throughput capability is competitive with large scale robotic batch processing.

III. INSTRUMENTATION AND METHODS

The core ACAPELLA sample processor embodies two essential concepts: (1) ACAPELLA uses glass capillary tubes to reduce sample size, minimize evaporation, automate the handling of small fluid samples, reduce thermal cycling times, and minimize the disposables needed to perform DNA sequencing. All processing steps are performed within these capillaries. Sample volumes are readily scalable from small (<1µL) to moderate (8 µL) sizes. (2) ACAPELLA uses piezoelectric subnanoliter dispensers (Engineering Arts, Mercer Island, WA) to add reagents to the capillary with high precision, essentially arbitrary low volume, and without any contact that might cause contamination.

A. ACAPELLA-5K Core Processor

The ACAPELLA-5K was designed with experience gained in the development and testing of the first generation ACAPELLA-1K system [5]. It extends the throughput of the 1K to higher throughput, while incorporating architectural changes that substantially improve the reliability and reproducibility of sample preparation. A photo of the ACAPELLA-5K is shown in Figure 1 and a functional schematic is shown in Figure 3. The ACAPELLA-5K has a circular architecture that drastically reduces the number of handoffs present in the ACAPELLA-1K system.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Automated Microfluidics for Genomics	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Department of Electrical Engineering University of Washington Box 352500 Seattle, WA 98195-2500	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-26, 2001 held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom., The original document contains color images.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
4

In the ACAPELLA-5K design (Figures 1,3), capillary handoffs are limited to the very beginning and very end of the process. Empty capillaries are dispensed from a "hopper," with integral fiber optic sensors to minimize mishandling and to provide feedback for error handling. From the hopper, each capillary is immediately inserted into a chuck, where it remains throughout the rest of the fluid dispensing and measurement process. Only at the final stage is it removed from the chuck, and transferred to the next step of the processing pipeline.

To process a DNA sample in the core processor, the sample is aspirated from a microplate well with a novel piezoelectric aspirator/mixer actuator, reagents are dispensed into the end of the capillary with piezoelectric reagent dispensers (Engineering Arts, Mercer Island, WA), all fluids inside the capillary are mixed with the piezoelectric aspirator/mixer actuator, and the capillary is off-loaded to a capillary cassette for further processing.

For details of the specific subsystems on ACAPELLA, see [6]. One important difference to note is that the aspirator and mixer in the ACAPELLA-1K system have been combined into one device on the ACAPELLA-5K system. This chuck is a novel device with a piezoelectric disk scavenged from a tweeter speaker. The fluid is aspirated or moved back and forth by air volume displacement driven by the piezoelectric actuator. (see [7] for a model of a version of this device). Currently the aspirator/mixer chuck operates in open-loop; a sensor is being integrated for precise feedback control.

For serial, pipelined processing, each step in the ACAPELLA-5K system must be under 5.76 seconds. Mixing of reagents can take from 3 to 15 seconds depending on the volumes and viscosities of the fluids being mixed [7]. Thus, in the ACAPELLA-5K system, mixing has been parallelized; mixing can occur while a capillary is held in an aspirator/mixer chuck and moved through three stations or up to 17.28 seconds (see Figure 3).

For DNA sequencing applications, the next steps are typically thermal cycling, DNA purification, and then loading of the samples to electrophoresis systems for analysis. Automated modules for these processes are currently under development.

B. Thermal Cycling Module

For reactions such as the Polymerase Chain Reaction (PCR) and sequencing reactions, it is necessary to cycle DNA samples typically through 3 temperature ranges (e.g. 94, 72, and 55 degrees C) per cycle, for 30 cycles total. Standard commercially available thermal cyclers take 2 to 4 hours for this process [2]. To accommodate the 5,000 sample/8-hour throughput of the ACAPELLA-5K, a faster thermal cycler is being developed in-house. Two small thermoelectric heater/cooler devices or Peltiers sandwich 48 capillaries held in a cassette (Figure 2). The low mass of the glass capillaries combined with the fast heating/cooling of the Peltier elements enable thermal cycling times on the order of 15 minutes total for 30 cycles. To maintain the 5K rate, 12 of these peltier-based thermal cyclers will cycle cassettes in parallel. A robotic arm will take cassettes from the off-loading station of the ACAPELLA-5K core processor and load them into 1 of 12 available thermal cyclers. After thermal cycling the capillary cassettes will move on a track to the purification module.

IV. Discussion

In this paper we report on the on-going development of an automated fluid processing system for biotechnology and chemistry. The initial concepts and proof-of-concept for this system were presented in [4] and the first generation ACAPELLA-1K processor was presented in [6]. The ACAPELLA-5K processor presented here, capable of processing 5,000 samples in 8 hours, has been built and is undergoing initial testing. New modules for thermal cycling and DNA purification will be integrated with the main processor for increased automated capability.

Acknowledgments

The authors thank Dr. Maynard Olson and the UW Genome Center for many consultations, helpful discussions, and generous collaborations in running experiments. We gratefully acknowledge the NIH NHGRI for their financial support of this research (grants # R01 HG01497 and R24 HG02215).

References

[1] F.S. Collins, "The Human Genome sequencing project," *Automation in Mapping and DNA Sequencing Conference*, Cambridge, England, 1999.

[2] D.R. Meldrum, "Automation for genomics, part one: preparation for sequencing," *Genome Research*, Vol. 10, No. 8, pp. 1081-1092.

[3] D.R. Meldrum, "Automation for genomics, part two: sequencers, microarrays, and future trends," *Genome Research*, Vol. 10, No. 9, pp. 1288-1303.

[4] D.R. Meldrum, "A biomechatronic fluid-sample-handling system for DNA processing," *IEEE/ASME Transactions on Mechatronics*, Vol. 2, No. 2, pp. 99-109, 1997.

[5] D.R. Meldrum and S.E. Moody, "Automating high-throughput fluid sample handling for biotechnology and chemistry," *Proceedings of the 2000 IEEE International Conference on Robotics and Automation*, San Francisco, CA, April 2000, pp. 198-205.

[6] D.R. Meldrum, H.T. Evensen, W.H. Pence, S.E. Moody, D.L. Cunningham, and P.J. Wiktor, "ACAPELLA-1K: A biomechatronic fluid handling system for genome analysis that processes 1000 samples in 8 hours," IEEE/ASME Transactions on Mechatronics, Vol. 5, No. 2, pp. 212-220, 2000.

[7] H.T. Evensen, D.R. Meldrum, and D.L. Cunningham, "Automated fluid mixing in glass capillaries," *Review of Scientific Instruments*, Vol. 69, No. 2, pp. 519-526, 1998.

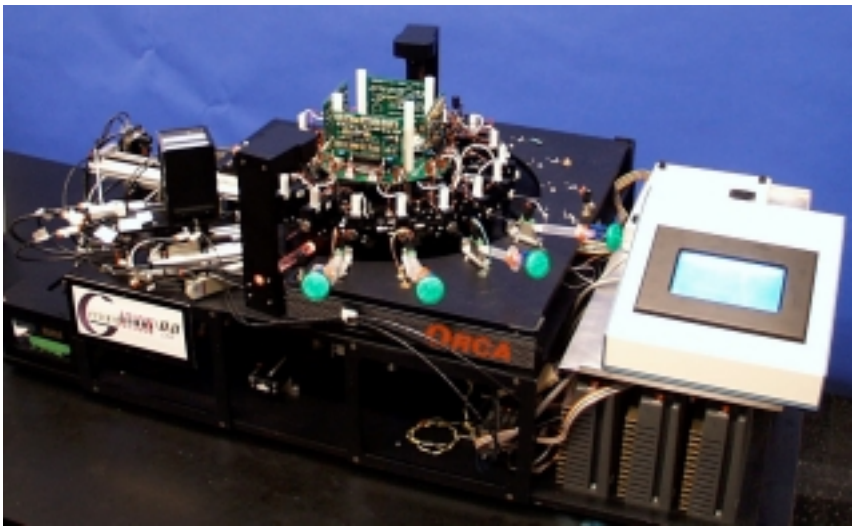

Figure 1. ACAPELLA-5K automated fluid sample handling system that processes 5,000 samples in 8 hours.

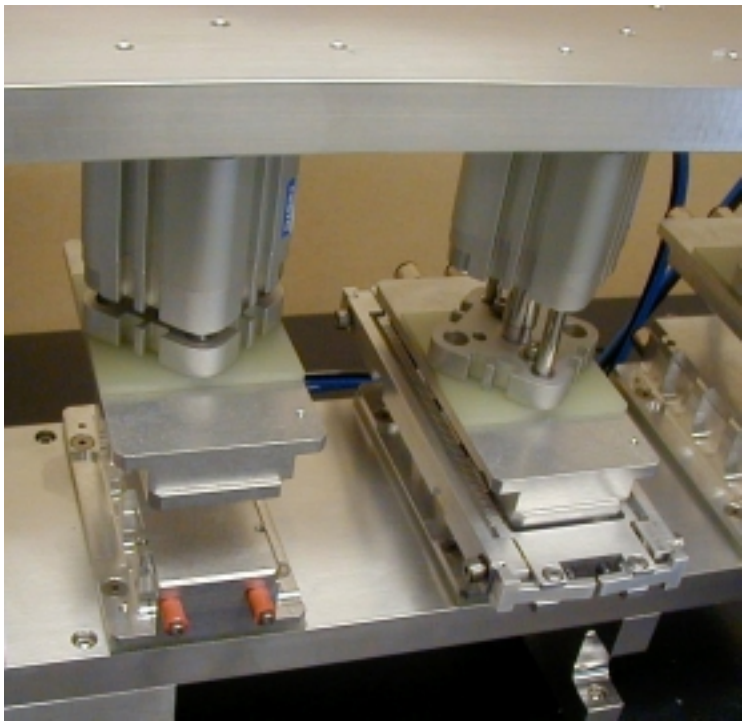

Figure 2. Peltier thermal cycler for handling cassette-sized batches of 48 glass capillaries with samples.

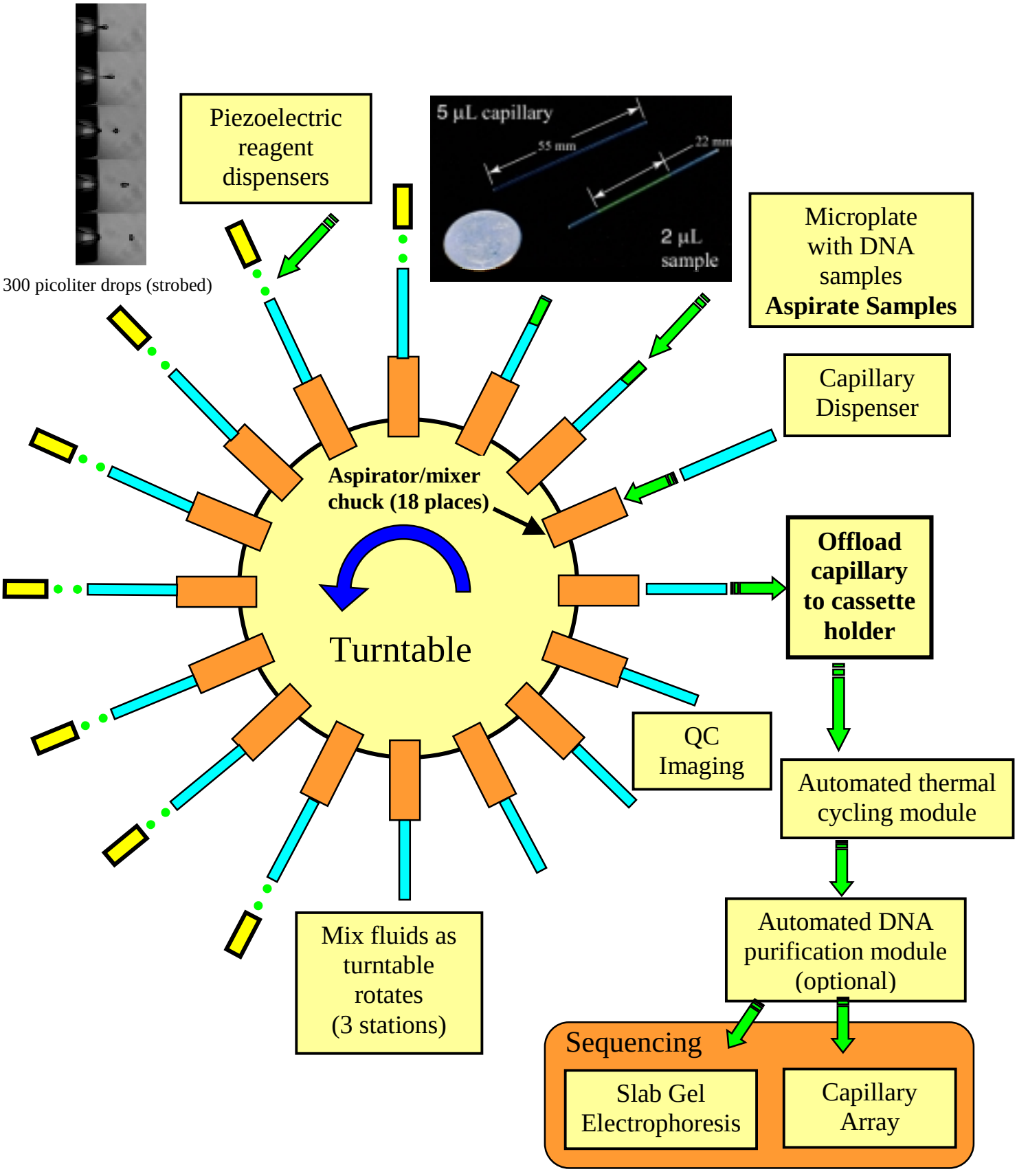

Figure 3. Functional schematic of fluid sample handling with the ACAPELLA-5K automated system.