CELLULAR GENOMIC ANALYSIS WITH GMR SENSOR ARRAYS

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ABSTRACT

A new cellular genomic analysis device is introduced that integrates cell lysing, microfluidics, and micromagnetic mRNA labeling and detection on one microfabricated substrate. The magnetic sensor, benzocyclobutene (BCB) microfluidics, investigative genomic assay, magnetic labels, and surface chemistry development for the new cellular genomic analysis device are described.

KEYWORDS: biosensor, genomics, GMR, magnetic labeling

1. INTRODUCTION

A number of issues hinder the genomic analysis of heterogeneous mixtures of cells. For example, current gene array technologies perform poorly when screening a low cell population. This limitation becomes even more relevant when dealing with the quantification of very low mRNA copy numbers per cell [1]. We have previously developed a unique DNA hybridization detection system that relies on giant magnetoresistive (GMR) sensors [2,3]. This system, based on the detection of magnetic microbead labels, is capable of observing a single DNA hybridization event. We are now attempting to apply this technology to the detection of low concentrations of mRNA, ideally that from a single cell.

0-9743611-0-0/μTAS2003/$15.00©2003TRF

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2003		**00-00-2003 to 00-00-2003**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Cellular Genomic Analysis with GMR Sensor Arrays	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Naval Research Laboratory,4555 Overlook Avenue SW,Washington,DC,20375**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
7th lnternational Conference on Miniaturized Chemical and Biochemical Analysts Systems, October 5-9, 2003, Squaw Valley, CA

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15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**4**	

2. DEVICE DESIGN

A unique microchip has been fabricated that includes dual field-in-plane excited spin-valve GMR sensors, microfluidic channels with a cross-sectional area approximately 140 μm^2, and micron-scale lysing electrodes (based on Figure 1). The fluidic channels are

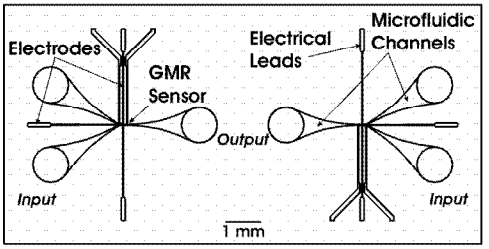

Figure 1. The layout of a GMR sensor chip with integrated microfluidic channels and lysing electrodes. The GMR sensor is approximately 180 μm long (not visible at this scale).

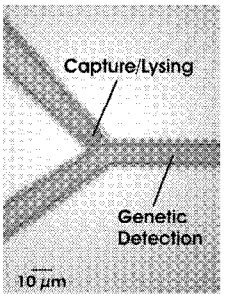

Figure 2. Two-layer BCB polymer microfluidic channels. The image is centered at the junction of the cell lysing and genetic detection zones.

built up from two layers of benzocyclobutene (BCB), a commonly used photosensitive polymer used in the electronics industry (Figure 2), and later sealed with a poly(dimethylsiloxane) (PDMS) cap. The fluidic system contains two distinct zones through which biological samples will be processed: 1) isolation/lysing and 2) genetic detection. The isolation/lysing zone contains a pair of electrodes at which individual cells can be electronically lysed using a series of high current density voltage pulses. The cell lysing region is connected to the labeling and genetic detection region by a shallow "filter" that is about 2 μm deep. For genome analysis, immobilized DNA or peptide nucleic acid (PNA) probes will cover the GMR sensors to create a planar capture surface.

Detection of genomic material occurs at a single GMR resistor that spans the entire detection region. The detection region is defined by microfluidic channels covering the

GMR chip surface. The detection region is about 10 μm wide by 180 μm long by 14 μm high. There is an identical reference resistor adjacent to the sense resistor that is not under the flow channel. It is expected that the detector will be able to see the entire range of 1 to 500 magnetic beads immobilized on its surface (based on 2.8 μm diameter commercial magnetic beads). The linear, low-hysteresis GMR sensing material is made using the following basic structure (listed from bottom to top, all dimensions in nm):

Ta 4 / NiFeCo 5 / Ta 5 / NiFeCo 4 / CoFe 1 / Cu 2.5 / CoFe 42 / CrPtMn 32.5.

This magnetoresistive material design generates about 5% resistance change over a range of +/- 20 Oe. (Several annealing steps are required to get ideal sensing behavior from these sensors.) The GMR sensors are covered with a 200 nm layer of Si_3N_4 for electrical isolation, and then common microfabrication techniques are used to form Al connections from the GMR sensors to bonding pads.

3. SUPPORTING EXPERIMENTS

Assay development. In preparation for examination of mRNA in raw yeast cell samples, we have developed reliable protocols with commercially available mouse brain and *Saccharomyces cerevisiae* poly(A)$^+$ RNA samples. These experiments are also leading to a calibration system that will be used to corroborate mRNA capture and detection via GMR sensing of magnetic beads. In addition, we plan to use these "idealized" samples of poly(A)$^+$ RNA to prove that our sensor is capable of low mRNA abundance detection without RT-PCR.

Magnetic microbead label development. Small lots of NiFe powder were vigorously shaken over a polycarbonate filter (5 μm pore) for several hours. The collected beads showed a tight distribution with an average diameter of 1 μm. Beads were coated with aminopropyltrimethoxy silane under drop-wise addition to a vigorously stirred solution. The amine-terminated beads were reacted with NHS-PEG-vinyl sulfone. Thiolated antibodies attach to the vinyl sulfone terminus to produce the conjugated magnetic bead.

Sensor substrate surface chemistry development. It was determined that more repeatable results are obtained for nucleic acid assays when using alumina rather than Au films as an immobilization substrate. The number of primary amines available after the addition of polyethyleneimine (PEI) and the number of vinyl sulfone (VS) groups present after the subsequent reaction with NHS-PEG-VS are very consistent when utilizing evaporated alumina layers. In addition, a blocking strategy involving the combination of casein and Tween® 20 has proven to be the most effective against non-specific binding of streptavidin-conjugated beads on the VS terminated surfaces.

4. CONCLUSIONS

A new GMR sensor and integrated BCB microfluidic channel design has been fabricated. In conjunction with supporting genomic assay, magnetic label, and biologically compatible sensor substrate surface chemistry development, this cellular genomic analysis system shows the promise of achieving the high sensitivity necessary for low mRNA abundance detection without RT-PCR.

ACKNOWLEDGEMENTS

This work has been supported by ONR, NIH/BECON and NIGMS, DOE, and DARPA.

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