Integrated Mass-Fabrication of Microfluidics for Diagnostic Chips

Magnetics-based concentration and detection

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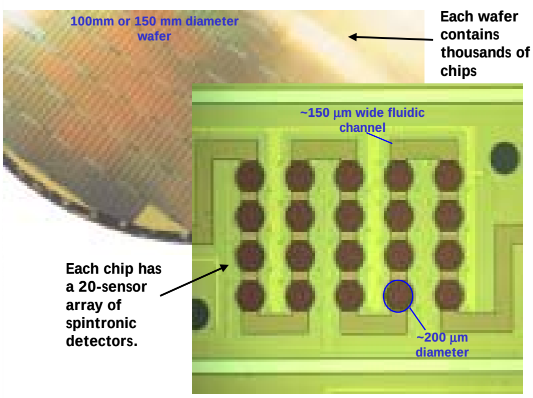
Abstract

Sealed microfluidic channels have been produced using a wafer-level fabrication process which yields thousands of finished microscopic biosensor parts on each wafer. The biosensors are arrays of magnetic detectors which measure the surface concentration of specifically bound magnetic nanoparticles in the detector field of view. The magnetic nanoparticles serve as an assay label, and can also be used to concentrate and manipulate analyte on the microchip level. A single detector in the array typically has a diameter of 100 microns, though they can be made to be less than 1 micron. Because the sensor encapsulation process happens during the wafer fabrication process rather than in a subsequent manual operation, these sensors can be made in vast quantities at a low cost per part. The detector arrays are subsequently incorporated into a larger card-sized fluidics cartridge that performs larger-volume microfluidic functions. These detector arrays have been used to detect femtoMolar concentrations of DNA with a dynamic range better than 3 logs. They are designed for disposable point-of-use applications where the material cost must be very small. The magnetic detection format also has the advantage of being rugged, small, and portable.

Solving the Micro- to Macro- fluidics interface problem

All microchip-based biodetection technologies need to be connected to fluidic interfaces that are of a much larger scale (e.g. µm to cm). This can mean higher cost and complexity. Work shown here is directed at developing a modular fluidics assembly approach. Ideally, macroscopic plastic fluid handling components will be manufactured using injection molding; microsensor and microactuators will be manufactured using semiconductor-based techniques. Interfaces will be a combination of MEMS and new manufacturing practices. The goal is to be able to manufacture these disposable devices in large quantities for about $1.

1) Wafer-level microsensor fabrication

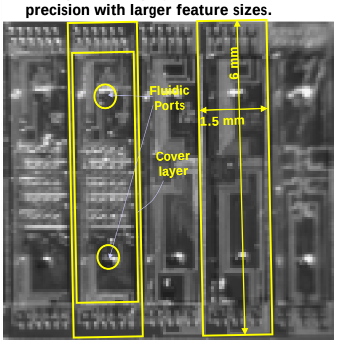


Each wafer contains thousands of chips

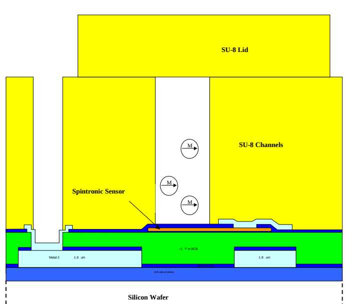
Each chip has a 20-sensor array of spintronic detectors.

2) Wafer-level fluidic cover and ports

Photopatterned polymer microfluidic channels combine high mechanical precision with larger feature sizes.

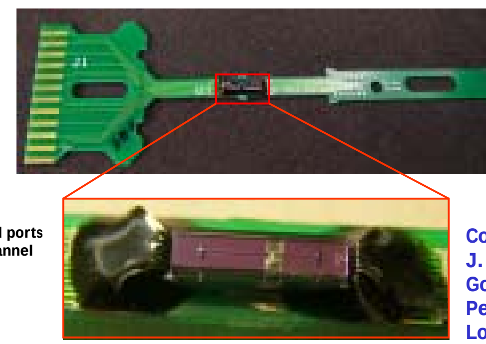


Close-up photo of 5 dice on the wafer.



Cross section diagram of wafer scale sensor and microfluidic channel fabrication process

3) Capped sensor dice are mounted on printed circuit board



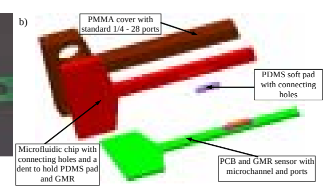
Chip is on disposable printed circuit board

Two 100-µm diameter fluid ports in an SU-8 channel cover

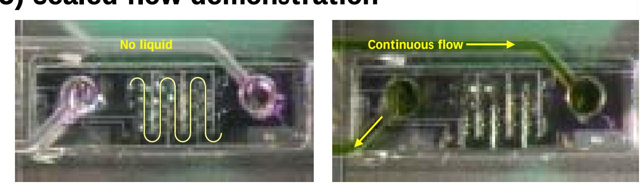
Collaborators at LSU; J. Hormez, J. Gottert, K. Lian, Z. Peng, M. Zhang, R. Louis; designed and made this interface

4) Plastic cartridge is sealed to sensor chip and board

Finished device with 2 standard HPLC tubing interfaces.



5) Sealed flow demonstration



Green liquid is pumped through to demonstrate flow and sealing.

NVE

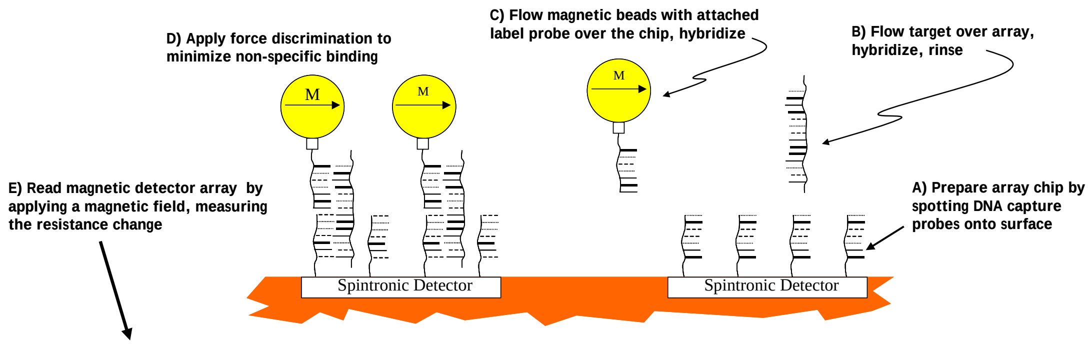
Magnetic labels enable integrated detection

Assays labeled with magnetic beads are read using disposable integrated detector chips. Associated reader modules are simple, compact and rugged. These advantages are attractive for fieldable diagnostic applications like food and water safety, homeland security, and remotely stationed military units. Magnetic labels can be used in place of many other label types including fluorescent, chemiluminescent, and radioactive reporters. They offer an ideal combination of high precision (single bead detection is possible) and simple detector hardware.

Example: two-probe DNA assay using 2.8 um magnetic beads

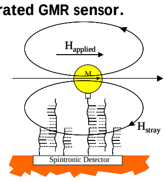
This assay mode was developed by L. Whitman et. al., NRL Code 6177

1) Number of magnetic labels on chip is proportional to concentration of DNA in sample



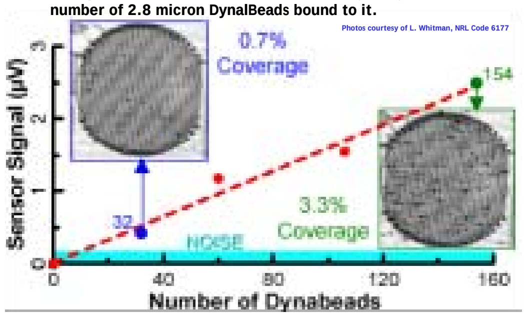
2) Magnetic labels are quantified using integrated magnetoresistive sensors

An applied magnetic field induces a magnetization in the magnetic bead. The stray field from this induced magnetization is detected by the integrated GMR sensor.



Detector sees $H_{Total} = H_{applied} + H_{stray}$ along a designed sense-axis. In the example here, the stray field is opposite in direction to the applied field. The magnitude of the stray field is strongly dependent on the distance from the label. Labels can be easily detected within about 1 diameter, but the signal falls off as distance[3].

Each photo here is of a GMR detector having the given number of 2.8 micron DynalBeads bound to it.



The measured sensor voltage is indicated by the vertical axis. Each sensor is about 200 µm in diameter. The beads show up as black spots. This detector has a linear response of signal vs. number of beads over about 2 to 3 logs.

cBASS™ Prototype

The GMR detector array is encapsulated in a clear plastic cartridge. This module is inserted into the magnetic reader box.

3) Recent assay results (NRL)

Multiplexed two-probe DNA hybridization assays and immunoassays performed in as little as 10 min

DNA assays with single-stranded target in buffer performed at <10 fM with only 15 min hybridization

Immunoassays under development: protein targets at ~1 ng/ml (and improving)

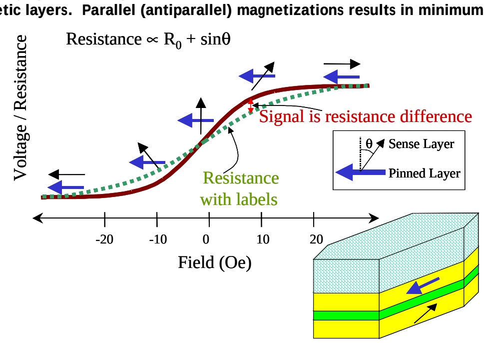
Simultaneous DNA and protein detection on one chip

Assays in complex sample matrices with minimal sample processing (e.g., whole blood plasma)

Giant Magnetoresistive (GMR) Sensors

GMR sensors are thin conductive films. Typically there are two ferromagnetic(e.g. Co) layers separated by a non-magnetic(e.g. Cu) layer. They are formed on silicon wafers, and patterned into long narrow resistor stripes using photolithographic techniques. The resistance is a function of the relative orientation of the ferromagnetic layers. Parallel (antiparallel) magnetizations results in minimum (maximum) resistance.

The green dashed curve represents the measured resistance when beads are present, and the solid curve is the initial resistance.



The magnetic films are designed so that one layer's magnetization remains fixed (thick blue arrow) while the other is free to rotate (thin black arrow) when a magnetic field is applied.

D.R. Baselt, G.U. Lee, M. Natesan, S.W. Metzger, P.E. Sheehan, R.J. Colton, A biosensor based on magnetoresistance technology, Biosens. Bioelectron. 13 (1998) 731–739.

D.R. Baselt, Biosensor using magnetically detectable label, US Patent 5,981,297 (9 November 1999)

R.L. Edelstein, C.R. Tamanaha, P.E. Sheehan, M.M. Miller, D.R. Baselt, L.J. Whitman, R.J. Colton, The BARC biosensor applied to the detection of biological warfare agents, Biosens. Bioelectron. 14 (2000) 805–813

J.C. Rife, M.M. Miller, P.E. Sheehan, C.R. Tamanaha, M. Tondra, and L.J. Whitman, Design and performance of GMR sensors for the detection of magnetic microbeads in biosensors, Sensors and Actuators A 107 (2003) 209–218

Approach to magnetic cytometry: detecting and manipulating magnetic labels in microfluidic channels

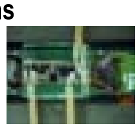
Magnetic sensors and actuators can potentially enhance or even replace optical and electrostatic technologies in cytometers. Magnetic beads have long been used to capture and concentrate dilute analytes and cells. This magnetic sorting technology has yet to be fully integrated into microfluidic systems. And the ability to detect single magnetic beads on magnetic sensor chips opens up many exciting possibilities in truly chip-scale detection applications. Results here are proof-of-principle demonstrations of on-chip detection and manipulation that point towards the feasibility of integrated detection of magnetically labeled cells. The next step in research will include actual cells and other biological analytes.

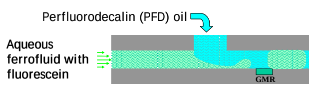
Detecting cell-like plugs of nanomagnetic particles in flow

In order to demonstrate the feasibility of detection of magnetically labeled cells using an on-chip Giant Magnetoresistive (GMR) magnetic sensor, a non-biological surrogate cell has been created. Plugs of ferrofluid have been created in a microfluidic flowstream that passes over a magnetic detector. These results show that the detection challenges of the integrated magnetic cytometer are technically manageable.

1) Microfluidic magnetic plugs form by merging magnetic and non-magnetic streams

Photo of PDMS cover over chip





2) Plugs flow over magnetic detector

Excitation field 15 Oe
Flow rate 250 nL/min
1.2% magnetite v/v

Plug dimensions:13 μm wide 18 mm deep 85 mm long

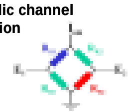
Ferrofluid composition: FerroTec 307 10nm Co particles

Two GMR detectors separated by 100 μm

Detectors ~ 4 μm wide

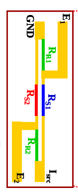
Plug volume ~20 picoliters

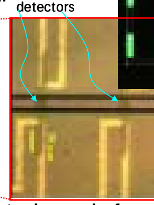
Plugs fill the entire microfluidic channel cross section



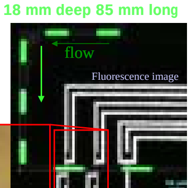
Four GMR resistors make up the integrated flow detector

GMR Magnetic detectors





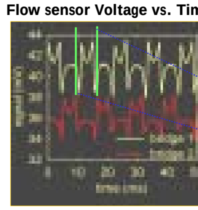
Photomicrograph of sensors prior to plug flow



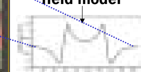
Fluorescence image of plugs in channel flowing over the GMR sensors

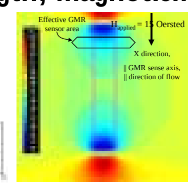
3) Quantifies plug velocity, length, magnetism

Flow sensor Voltage vs. Time



Predicted Volts vs. time based on magnetic field model

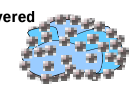




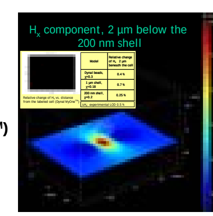
Magnetic field model of plug

4) Model prediction for cell detection

Hypothetical cell covered with magnetic labels



FEMLAB package
Protozoan cell 8x6x6 μm
48 1-μm spheres, χ=0.3 (Dynal MyOne™)
Homogeneous 1-μm shell, χ=0.18
Homogeneous 200-nm shell, χ=0.2
(Micromod nanomag-D, χ=2)



Pekas et al., Appl. Phys. Lett., 85, (2004)

T. Thorsen, R. W. Roberts, F. H. Arnold, and S. R. Quake, Phys. Rev. Lett., 86, 4163 (2001)

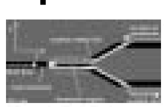
H. Song, J. D. Tice, and R. F. Ismagilov, Angew. Chem. Int. Ed. 42 (7), 768 (2003)

Magnetophoretic sorting of magnetic labels

A uniform external field magnetizes particles

Current lines induce field gradients of 10^2-10^3 T/m

Resulting force diverts particles to a desired channel



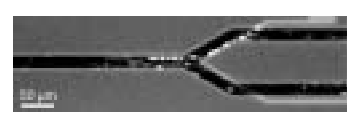
Top view

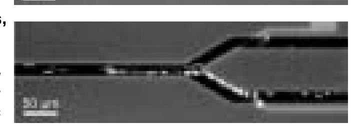
Cross section diagram

Magnetic particles reach a cross-channel terminal velocity of about100 μm/s in about 100 ns (assume 1 μm diameter, χ=0.18)

Sorting is about 85% efficient in these photos, design improvements should increase this.

The next generation of magnetic flow sorters will be used to send magnetically labeled cells one way and unbound magnetic labels another

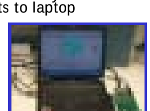




Photos of 2.8 micron Dynal beads (w / fluorescent dye) deflected left or right depending on + or - electrical current

Assay Reader Hardware

Magnetic field source and electromagnetic measurement system connects to laptop



Sensor chip mounted on printed circuit board: DISPOSABLE, plugs into reader module

Nikola Pekas, Marc Porter, et. al. in the Chemistry Department at Iowa State University contributed greatly to this work

"Magnetic particle diverter in an integrated microfluidic format," Pekas, N., Granger, M., Tondra, M., Popple, A. and Porter, M. D, Journal of Magnetism and Magnetic Materials, 293, pp. 584-588, (2005).

"Design of Integrated Microfluidic Device for Sorting Magnetic Beads in Biological Assays," **M. Tondra,** M. Granger, R. Fuerst, M. Porter, C. Nordman, J. Taylor, and S. Akou, IEEE Transactions on Magnetics 37, (2001), pp. 2621-2623.

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