Integrated Mass-Fabrication of Microfluidics for Diagnostic Chips

Magnetics-based concentration and detection

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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 fluids 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.

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
2) Wafer-level fluidic cover and ports
3) Capped sensor dice are mounted on printed circuit board
4) Plastic cartridge is sealed to sensor chip and board
5) Sealed flow demonstration
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 magneto resistive 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$^3$.

3) Recent assay results (NRL)

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

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

Immunosassays 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 Magneto resistive (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 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.
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

2) Plugs flow over magnetic detector

3) Quantifies plug velocity, length, magnetism

4) Model prediction for cell detection

Magnetophoretic sorting of magnetic labels

Assay Reader Hardware

Photo of PDMS cover over chip

Photo of plugs flow over magnetic detector

Photo of sensors prior to plug flow

Magnetic field model of plug

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

Photo of magnetophoretic sorters will be used to send magnetically labeled cells one way and unbound magnetic labels another

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