# Micro-electro-fluidic module to control magnetotactic bacteria for micromanipulation tasks under an optical microscope

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## ABSTRACT

This project describes a Multi-Chip Module (MCM) that contains a microelectronic circuit and a microfluidic device that could be combined to implement a "bacterial microfactory". The microchip contains two decoders connected to arrays of horizontal and vertical wires respectively, forming a matrix used to process commands received from an external computer. The electrical current flowing through the matrix is generated from internal voltage-to-current converters. The electrical current circulating through a metal conductor generates a magnetic field that is used to guide the movement of Magnetotactic Bacteria (MTB) in the microfluidic device. The dedicated microfluidic device is micro-fabricated on a glass wafer. Preliminary results show that a single MC-1 MTB can push a 2 µm microbead at speeds reaching 100µm/s under the control of an external magnetic field of less than 10 Gauss. A Carl Zeiss microscopy software (AxioVision) is used to control and configure the Axio Imager Z1 optical microscope and allows us to develop customized plug-in with Visual Basic for Application (VBA). The control electronic die was hence programmed as a VBA module, simplifying interoperability between the control, data recordings and microscopy observations. The parallel port of an Intel Pentium 4, 3.0 GHz equipped with 2.87 Go of RAM running Windows XP was used to communicate with the circuit. Connected to the parallel port, two demultiplexers interface the chip and the port. Patterns to control the bacteria such as left-right and up-down displacements were implemented and tested. Other more complex patterns to capture, attract and repel the bacteria from the center of the chip were also designed and validated.

**Keywords:** Magnetotactic bacteria, microfactories, microfabrication, hybrid microsystem, multi-chip package,

## 1. INTRODUCTION

The control Magnetotactic Bacteria (MTB) [1, 2] is achieved by inducing a torque on a chain of membrane-based nanoparticles called magnetosomes being embedded into each bacterium. The chain of magnetosomes acts like a compass. In previous works, we have demonstrated the controllability of the MTB [3, 4]. Here, we present a platform to control the motion of the MTB that are to be used as actuators for the manipulation and assembly of micrometer-sized objects. The CMOS microelectronic chip is used to generate the magnetic field lines to orient the swimming direction of the MTB, while the microfluidic chip is in direct contact with a matrix of conducting wires in a micro-reservoir (or in a drop of an aqueous medium retained by capillary forces) that contains the MTB. With the use of a microscope and special custom software, the control of the MTB is automated. These results could lead towards bacterial microfactories, where micro-sized objects could be assembled and manipulated automatically. The CMOS and the microfluidic chips shown in Fig. 1 are interconnected using the Micro-Chip Module (MCM) method of assembly with a pre-defined layout. The resulting circuit is mounted onto a Printed Circuit Board (PCB) providing a communication interface with an external computer. The PCB is then placed under an optical microscope for tracking the bacteria during control.

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## 2. SYSTEM ARCHITECURE

With the conception of such a microsystem, we aim at controlling with precision, magnetotactic bacteria previously injected onto a microfluidic matrix, towards the realization of a bacterial microfactory with future applications ranging from simple micromanipulation to complex parallel controlled tasks. Some specific short range objectives are to effectuate manipulations on samples of bacteria in order to characterize them, and to design a circuit that would serve as a basis to carry out first order manipulations. This would allow us to evaluate the motility of the bacteria or assess the realization of tasks such as using the bacteria for surface screening or micro-handling of microbeads. This requires not only components such as an embedded microfluidic reservoir to gather the bacteria but also software for tracking the bacteria (with the assistance of an optical microscope), along with a user interface specially designed for conducting the experiments.

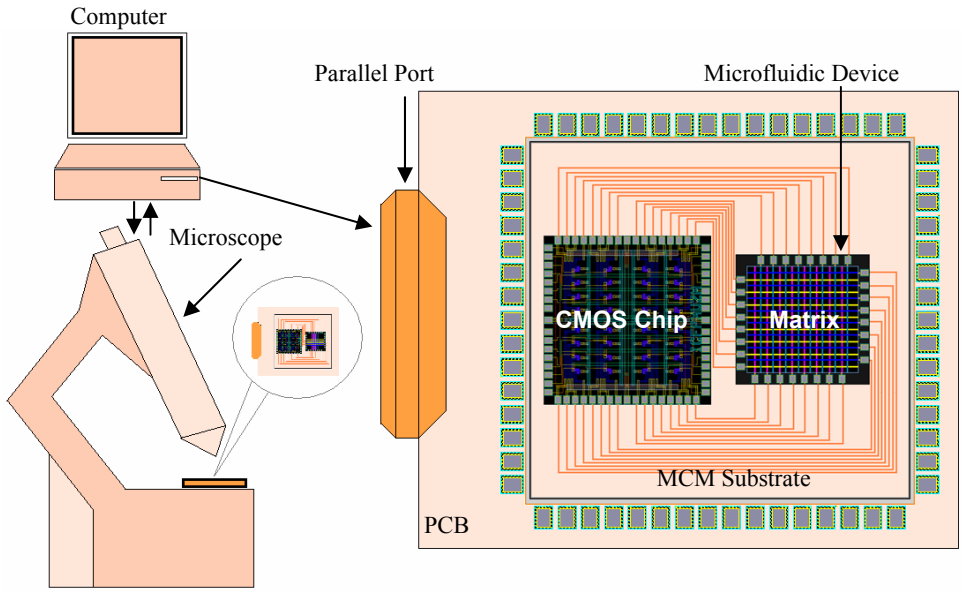



Fig. 1 System architecture.

Figure 1 shows the floor-planning and the routing of the units that compose the MCM. The total dimensions of the matrix are of 200 µm × 200 µm. The conducting wires have a width of 15 µm with a spacing of 10 µm between two adjacent conductors.

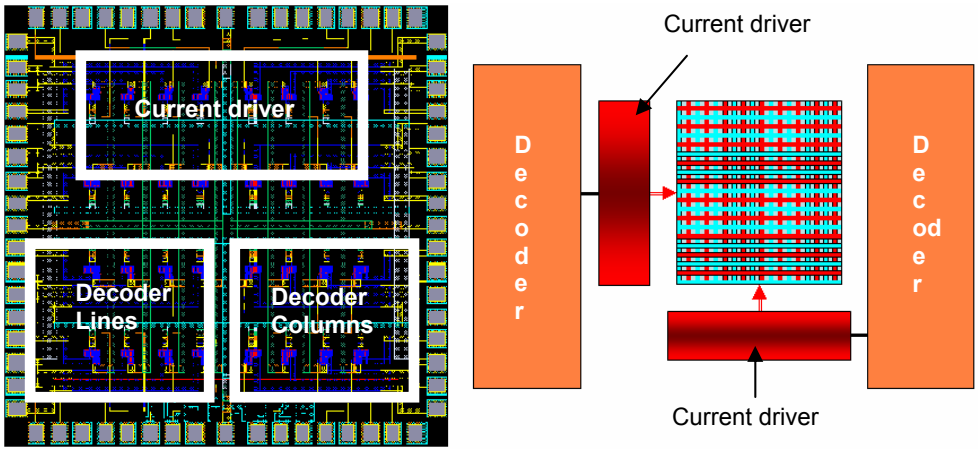
## 3. MICROELECTRONIC CIRCUIT TO CONTROL THE BACTERIA

The internal architecture of the microelectronic circuit controlling the movement of the bacteria consists mainly of two decoders to select the rows and the columns respectively within a matrix. When selected, an electrical current is injected for generating a local magnetic field that is used to orient the swimming direction of the bacteria. The current driver modules are responsible for the intensity of the current to be injected while special computer software allows autonomous operations. The magnitude of the magnetic field generated is predicted by the Biot-Savard law where

$$B = \frac{\mu_c I}{2\pi d} \tag{1}$$

In Eq. 1, $B$ is the magnetic field, $\mu c$ is the permeability of the conductor, $I$ is the intensity of the current to be flown in the conductor wire, and $d$ is the distance between the chain of magnetosomes in each bacterium and the conductor. According to this equation, the closer the bacteria are from the conductor, the more they will sense the magnetic field. The magnetic field generated will induce a torque on the chain of membrane-based nanoparticles (magnetosomes)

embedded onto each bacterium. The chain of magnetosomes will align with the magnetic field lines; resulting in controlled swimming directions of the bacteria. Accordingly, by injecting a current to selected conductors in the matrix, one can control the motion of the bacteria. Figure 2 (a) shows the microelectronic circuit dedicated to control the MTB obtained from Cadence Layout Virtuoso, while Fig. 2 (b) shows the internal architecture of the circuit.




(a) (b)

Fig. 2. Internal architecture of the microelectronic chip

In the present implementation, a current of 10 mA must be injected into the conductor to produce a magnetic field intensity of 2 Gauss, which is sufficient to control the motion of the bacteria placed onto the matrix conditional to the fact that the bacteria are kept within a distance of 10 µm from the conductor wire in a the worst case scenario. Lower current densities could be expected by decreasing the features size

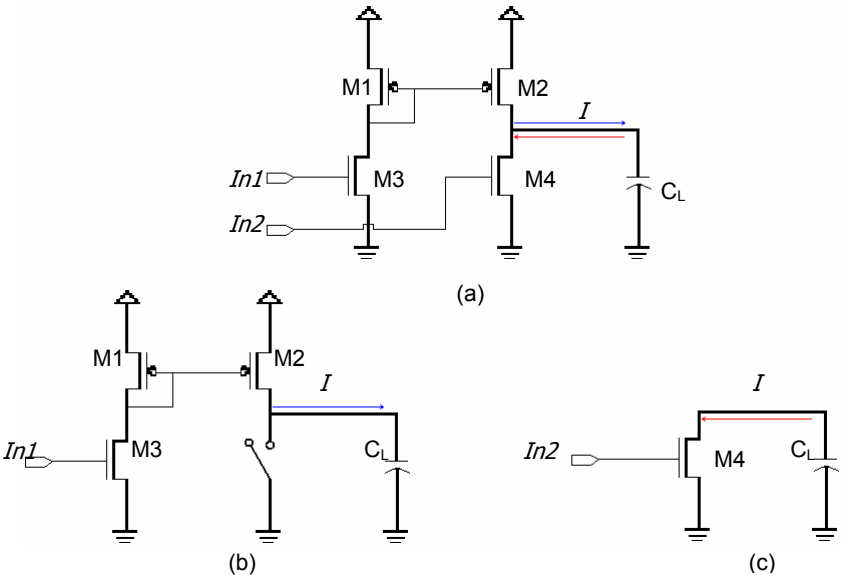



Fig. 3. (a) Circuit for the current driver to generate the magnetic field to navigate the bacteria. (b) Shows the current flowing into one direction for a particular motion of the MTB (c) shows the reverse direction of the current; therefore reverses the direction of the magnetic field.

Figure 3 shows the internal circuit architecture that is used to inject the current into the conductor. It is based on a modified current mirror circuit. Transistor M3 is the biased transistor, while transistors M1 and M2 form a mirror current while M4 is used to reverse the direction of the current in the conductor. When transistor M3 is on, it will be mirrored to the transistor M2 ($I_{M2}$), with transistor M4 off, $I_{M2}$ will charge the load capacitance as depicted in Fig. 3 (b). To reverse the direction of the current, we turn off transistor M3, and turn on transistor M4. In this manner, M4 will discharge the load capacitance as depicted in Fig. 3 (c). By reversing the current in the conductor, we reverse the orientation of the magnetic field lines generated, thus the direction of the trajectory of the MTB.

## 4. SOFTWARE

The software module is responsible for the detection and tracking of the bacteria to be controlled by means of image analysis and processing, and then to activate the proper conducting lines according to a desired pre-programmed motion pattern. For the moment, it operates in a semi-automatic fashion for the purpose of gathering experimental data and building expertise. Nonetheless, full automatic operation mode is also possible.

For the experiments, the first step consists of choosing the motion pattern to be applied. If the desired resulting motion is for example rectilinear to the left, then a correct current should be applied to the vertical conducting lines of the grid in the proper direction. The result would be a magnetic field guiding the bacteria in the desired left direction which its motility is being exploited for conducting mechanical tasks such as the manipulation of micro-objects. This practically converts to the simple click of a button. A series representing the basic motion behaviors have been programmed and are accessible through a software user interface. More complex motions are also possible and consist of mere combinations of the previously mentioned basic movement functions. The motion of the bacteria is visualized and recorded through the Couple-Charged Device (CCD) of the optical microscope and hence, a direct correlation would be established between the control applied to the matrix and the bacteria actual displacement patterns by comparing the timeframes of the acquired images and the control commands of the motion patterns.

For finer experiments, i.e. those dealing with a single bacterium, the experimenter must first select a specific bacterium to be tracked. This is done by letting the user to enter a selection mode and then click with the button of the mouse on a moving target. If the target displacement characteristics fall within range of the allowable tracking thresholds, then the first tracking cycle starts, at the end of which a calculation corrects the control of the grid lines (i.e. which lines to activate in what direction) according the desired projected position of the bacterium at the next cycle. Figure 4 depicts a typical cycle of execution.

The software was developed as a Visual Basic for Applications (VBA) extension to Carl Zeiss AxioVision Software. That way, taking advantage of the image processing libraries and controlling the microscope through its Object Model was possible.

The decoders' hardware interface specifies 5 data bits for the grid line or column and an extra bit for the decoder selection. Communication is done through the computer's parallel port which is accessible in VBA through widely available generic dynamic libraries. The rows and columns of the grid have been numbered from 0 to 63, the rows having the even numbers. A function have been written that takes as a parameter a row or column number and generates the corresponding series of bits to the parallel port following a simple mapping process.

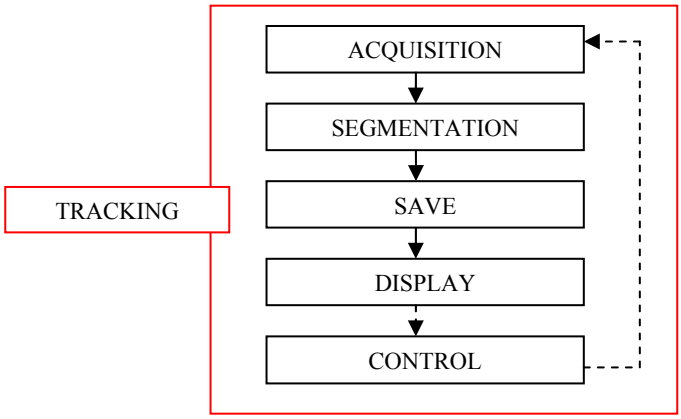



Fig.4 Typical experiment cycle

For ease of use, the control of the grid is mapped graphically onto the user interface. The resulting software user interface can be seen in Fig. 5. It includes control buttons for magnetic fields in the four directions as well as more complicated path patterns and general configuration controls.

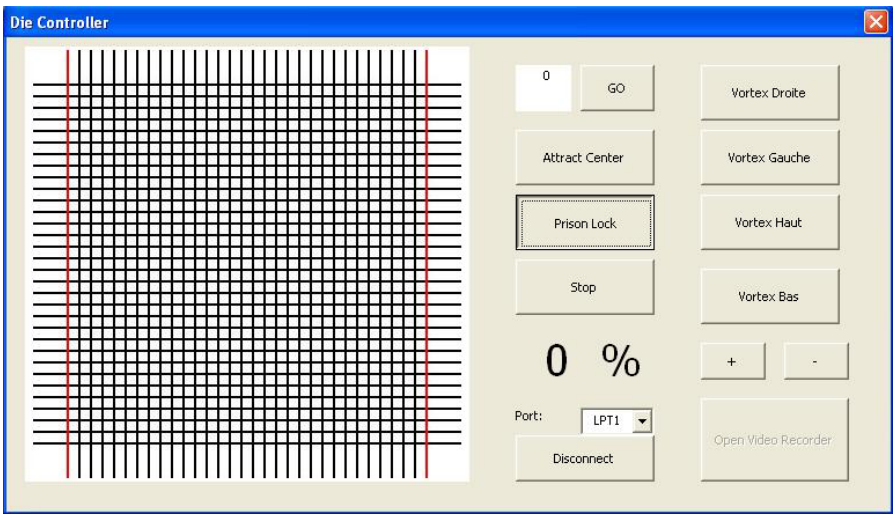



Fig.5 Screen capture of the control user interface

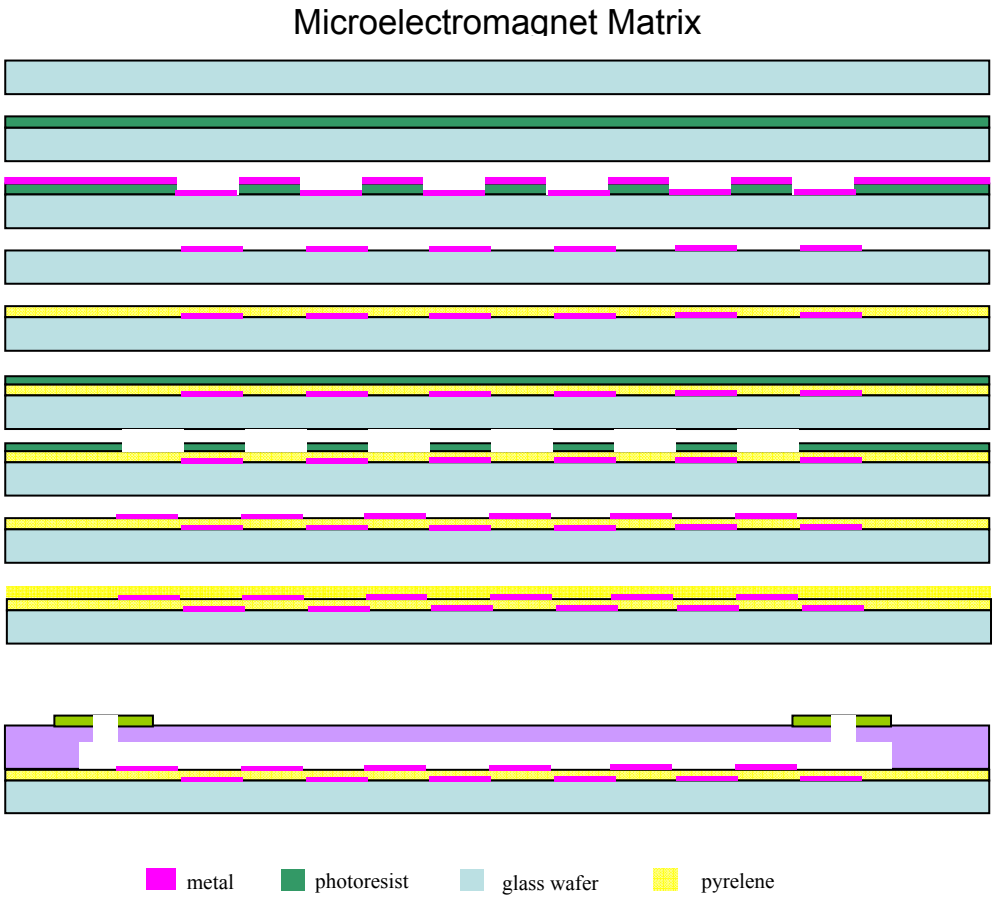
## 5. MICROFLUIDIC DESIGN

The dedicated micro-device is comprised of two components, the microelectromagnet matrix and the microfluidic component. The microelectromagnet matrix is used to generate local magnetic field to inject electric current into conductive metal wires. Connected with the CMOS chip, the flux and direction of magnetic fields generated by the matrix can be precisely controlled. The microfluidic part provides a microchamber on the top of the microelectromagnet matrix and outlets for bacteria medium injection.

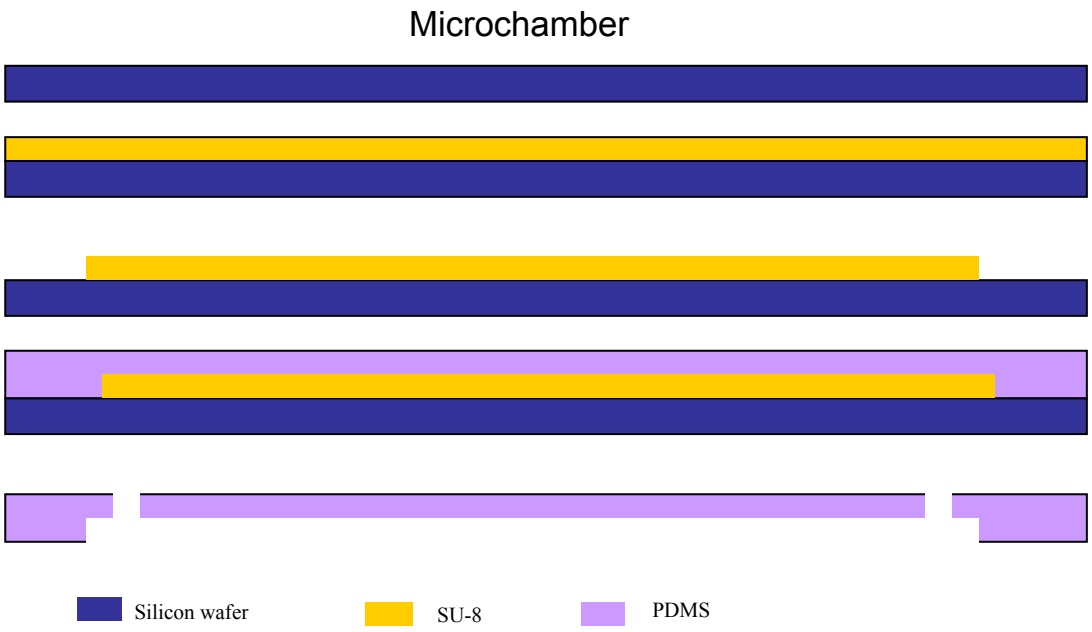
The microelectromagnets matrix has two layers of conducting wires and two insulating layer. Two isolated metal layers (Cr/Au) are deposited by electron beam deposition technique on a Pyrex glass wafer, then, patterned with conventional

lift-off technology. A piece of clean 4 inch Pyrex glass wafer is treated with AP300 to increase the adhesion of photoresist. After that, 2 µm thick photoresist (SP1813) is spin-coated and lithographically patterned to form the conducting wires. Then, a 5 nm thick layer of titanium is deposited on the Pyrex substrate as a seeding layer followed by the deposition of a gold layer with a thickness of 1 µm. Finally, photoresist and unwanted metal are removed from the substrate. Before the deposition of second layer of conducting wire, a 2 µm thick dielectric parylene layer is deposited on the first layer of metal to form an insulating layer, which prevents the electrical short between wires. After the deposition of the second metal layer, which includes conducting wires and bonding pads as well, additional layer of parylene is deposited to insulate the top metal layer and provide a surface for bacteria manipulation. The detailed fabrication steps are illustrated in Fig. 6.

The microfluidic device is mainly made of PDMS using soft lithography [5]. Photoresist SU-8 is utilized as a mold to construct the microchamber. SU-8 photoresist is spin-coated and patterned on a silicon substrate with standard lithography technology, then, PDMS is poured on the surface of photoresist. After the curing process, the PDMS is peeled carefully from the silicon substrate. Outlets (2mm in diameter) are punched on the PDMS. Followed by the oxygen plasm surface activation process, the PDMS microchamber is aligned and bonded with the glass wafer to finalize the fabrication process. Figure 6 describes the fabrication steps.




(a)




(b)

Fig. 6. Fabrication steps of (a) microelectromagnets and (b) microfluidic sections.

## 6. CONCLUSION

Flagellated bacteria are known to be effective bio-actuators in comparison with other actuation methods in microsystems. In particular, MC-1 magnetotactic bacteria can provide thrust force exceeding 4 pN per bacterium while being controllable through magnetotaxis using a computer where a torque generated by a small electrical current can be used to generate local magnetic fields to orient the swimming direction of the bacteria. By exploiting the motility of the flagellated bacteria acting under computer control, lower electrical power with added flexibility for manipulating small objects can be realized. Here, we presented a platform that could be used as a micro-factory for assembling small objects in a fluidic environment. The process has the advantages of not requiring high voltage as other manipulation techniques such as dielectrophoresis do, being more compatible with the low voltage levels typically encountered in CMOS microelectronics, while being independent of dielectric properties. As such, ths paper showed an example of a platform where bacterial manipulation is considered as an alternative for the implementation of micro-factories.

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