Manipulation tools for biological applications using microrobots with nano-range accuracy.

Josep Samitier*; Manel Puig-Vidal*; Jaume López*; Pere Miribel*; Jose Mª Lopez-Villegas*, Ramon Casanella, Cristina Madrid, Antonio Juárez.

Bioelectronic and Nanobioscience Research Center (CBEN)
University of Barcelona. C/ Martí Franquès, 1. 08028 Barcelona.

ABSTRACT

The development of tools for a multi-microrobot manipulating system prototype to handle cells and biomolecules is proposed. The system will be based on a cluster (5 to 10) of small (cm³) mobile autonomous robots, comprising several essential subsystems such as a global positioning system to provide accurate position information of each microrobot and advanced manipulating tools for biological applications. Actuators for the positioning and moving of cells must be compatible with their living conditions. The application of non-uniform electric fields to non-charged particles suspended in aqueous medium produces a force over the particle due to the induced dipole moment

Keywords: Micro-robots, piezo-actuators, dielectrophoresis, cell handling

1. INTRODUCTION

The progress of Micro Systems Technology has led to a renewed interest in fabricating robots for autonomous tasks in applications fields ranging from mechanical handling to biology and medicine /1/ . Transport and manipulation of biological cells or assembly of micro mechanical parts are presently made by large high-precision application specific instruments and exchanging these instruments with miniaturized robots should not only be advantageous from a size and flexibility point of view /2/, but has also a potential to give improved performance at reduced fabrication costs. Two examples of miniaturised robots with various degree of integration are the Nano-Walker /3/ and the dm-sized MINIMAN-robot /4/. Very recently, the authors in collaboration with the Department of materials Science from Uppsala University, have presented a miniature microrobot (dimensions around one cm³) driven by smart power integrated circuits. /5,6 /

In the framework of the MICRON IST European project IST-2001-33567, the goal of the consortium is to develop a system based on a cluster of such small (cm³) mobile autonomous robots. They will consist of a locomotion system allowing high velocity combined with a large working area and nanometric resolution. The robots will navigate on a flat, horizontal surface by translation and rotation. The complete system design will enhance the performance achievable by each individual microrobot, by suitable distributing various mechanical degrees of freedom among many microrobots to enable complex object handling and manipulation.

As in most miniature robots /3,4/ piezoceramic actuators are used in the present work. This material is chosen since it offers good performance with a technology that is mature and able to give high reliability components at a reasonable cost. Small steps can be produced at a high frequency resulting in as well high precision as high handling speed. Nevertheless, to achieve large strain a high electrical field has to be applied to the material and typically, high voltages have been used so far. To reduce this high voltage biasing levels to levels feasible for integrated smart power circuits, different multilayer piezoceramics materials are used /7,8/.

As the main applications of MICRON robots are in the biological domain, additional bio-handling arms must be integrated. Actuators for the positioning and moving of cells must be compatible with their living conditions. The development of tools for biological scale (some microns in the case of cells and some nanometers in the case of biomolecules) requires two different approaches. On one hand, existing conventional micro-manipulating tools can be
further miniaturized. Alternatively, techniques for nano-manipulation, which currently employ SPM tips for physical interaction, will be adopted.

In the first approach, the application of non-uniform electric fields using microelectrodes, to non-charged particles suspended in aqueous medium produces a force over the particle due to the induced dipole moment. Depending on the electrode layout, the dielectric constants, the field frequency and phase, the polarized particle will move towards the highest electric field (positive dielectrophoresis), move away from the highest fields (negative dielectrophoresis), rotate or translate linearly as a consequence of a traveling electric field (traveling wave dielectrophoresis). In a similar way, handling and manipulation of cells can be performed using small size functionalized magnetic particles. Ferromagnetic microspheres previously magnetized using a high magnetic field or paramagnetic spheres submitted to a non-uniform magnetic field can be used /9/.

Using the capabilities of cantilever tips integrated in the microrobot arms, would be available simultaneously that the microrobot works as manipulator and sensing device. An SPM tip with attached molecules or molecule cluster (functionalised tip) is capable of picking up and transporting other molecules or particles. In this case the tip acts as a nano-gripper. Moreover, AFM is currently used in cells experiments to obtain image or mechanical properties studying the mechanical interaction between the tip and the cell (nanoindentation experiments). Taking into account that it is necessary to adapt and to integrate AFM functions in the MICRON microrobots platform, the first approach it to constrain the capabilities of the system to obtain local probe manipulation information.

In this framework, this paper explain the present development of cm$^3$ piezoactuator microrobots and the integration of micromanipulation tools. In addition some biological manipulation experiments using Escherichia Coli and Yeasts (Saccharomyces Cervisiae) are introduced.

### 2. PIEZOELECTRIC ACTUATORS FOR A MINIATURE ROBOT

The firsts cm$^3$-sized robot are based on two monolithic piezoceramic actuator units. A first prototype of the miniature robot is shown in figure 1.

![Figure 1: First prototype of a miniature robot for micromanipulation. Two piezoceramic actuator units are put together back to back. One actuator unit is intended for lateral movements on a flat surface and the other for rotating a ball including a tungsten probe.](image)

Each actuator unit has six multilayer piezoceramic elements monolithically integrated on a base, figure 2. The actuator elements are divided into thin layers, with alternating phase and earth electrodes in between. Using this multi capacitor configuration, the driving voltage can be reduced to a level which allow to use integrated circuits for driving /7,8/. The phase electrode layer of each actuator element is divided into four electrically separated elements.

In principle, each actuator elements works as bimorph element, but the partition into four independent quadrants makes that each leg acts as a three-axial actuator. Each actuator element can be extended longitudinally and bent in any lateral direction, depending on the voltage applied to the quadrants. Looking at a projection of the element tip movement into a plane parallel to the sidewall of the element, the tip can move along essentially any trajectory within a rhombic area (Figure 3).
Figure 2: (a) Six-legged monolithic actuator unit. The size of the whole platform is 1x1 cm². Each actuator element is divided into four electrically separated quadrants. Therefore, the tips of the actuator elements can move in three orthogonal directions. (b) Top view of the electrode pattern. (c) Cross section of one actuator element showing alternating phase and earth electrodes.

Figure 3: Cross section of an actuator element. Depending on the voltage supplied, the element tip can be positioned at any position within the rhombic area.

3. DRIVING SYSTEM

Since the monolithic actuator unit should be able to create a three-axial motion with high precision, it is necessary to define some type of driving system strategy related to the choice of power signal waveform. To achieve the desired type of movement high voltage signals with peak values of 50 V has to be supplied to the 24 quadrants per actuator unit. CMOS standard integrated technologies are not capable to withstand the required voltage levels, thus, driver circuits for piezoactuators are usually based on hybrid solutions using discrete components. Due to the size of these hybrid solutions, the driver unit cannot be placed on the actuator unit. As a consequence, the movement and precision of the tethered miniature robot, with more than 50 power wires in this case, would be seriously limited by the wires between the actuator unit and the driver unit.

An available commercial 1.2 µm BDC technology (Bipolar, DMOS, CMOS), HBIMOSF from MIETEC-ALCATEL, capable to withstand 80V, has been used used to design a specific on-board Smart Power Integrated driver circuit. Using this technology it is possible to integrate power and control circuitry. The digital circuitry to control the power driver is included to standardize all the drivers.
Considering the specification requirements, 10nm maximum accuracy, a 8-bits DAC converter has been included. The voltage signal waveforms are generated in an external control module and sent in digital format using 8 bits and 256 samples per period, by means of a Time Division Multiplexing (TDM) technique. Special precautions have been taken for the layout design in order to avoid substrate coupling effects between digital, low-voltage analogue and high-voltage power analogue blocks. The resulting ASIC size is $5600 \times 3300 \mu m^2 / 10/$. 

The driving platform consists of six Smart Power ICs mounted on a 2 layer printed circuit board with wire bonding interconnections (Fig. 5). Two identical driving platforms are needed to obtain a complete miniature robot. The final microrobot prototype obtained is depicted in figure 6.

**Figure 4:** Smart piezoactuator drivers architecture

**Figure 5:** Driving platform: 6 Smart Power ICs mounted on a PCB.
Experiments have been performed with the robot placed on a flat polished surface (flatness about 20 nm). Driving platform with a parallel control strategy were used. Linear motion in two orthogonal directions and rotation has been tested at different frequencies below 1KHz. The speed at a given frequency is typically about half the theoretical maximum speed, which can be explained by inhomogeneity factors such as small size differences of actuator elements.

4. TOOLS FOR HANDLING AND MANIPULATION

Different tools can be implemented in the microrobot platform for handling applications. Figure 7 shows a schema of three different approaches: a) microelectrodes to produce non-uniform electric fields, b) micro-coils to detect ferromagnetic spheres attached to the cells; c) micro-fabricated cantilevers to measure interaction forces with high resolution. In this last case, scanning probe microscopy is very useful to probe cell topography and study mechanical properties in physiological conditions. The usual resolution reported for living cells using commercial Atomic Force Microscopy is about 100nm, value compatible with the accuracy of the developed micro-robots.

Microelectrodes array are easy to include in the micro-robot arm (figure 7-a). The application of non-uniform electric fields to non-charged particles suspended in aqueous medium produces a force over the particle due to the induced dipole moment. Depending on the electrode layout, the dielectric constants, the field frequency and phase, the polarized particle will move towards the highest electric field (positive dielectrophoresis), move away from the highest fields (negative dielectrophoresis), rotate or translate linearly as a consequence of a traveling electric field (traveling wave dielectrophoresis).

In addition to the use of non-uniform electric fields for bioparticle manipulation, electric field traps can be used to confine particles in a desired region where they cannot escape unless an additional force is applied. The combination of these concepts with the sensing, manipulation and transportation capabilities of several, specialized mobile micro robots
which can easily be grouped around a narrow space to probe the properties of a biological specimen, opens up a completely novel domain of biological experiments.

Dielectrophoretic particle traps have been successfully used for many biological applications, after the first works reported by Pohl /11/. For instance we may mention: artificial-particle and bioparticle characterization /11/, devices for cell fusion /12/, particle separation based on its differential Polaris ability /13/, particle collection /14/, cellular culture over a field-protected electrode surface /15/, cell shift register and bio-cell deflector /16/, and particle handling in terms of linear motion and positioning /17/ levitation /18/, and trapping in electric-field cages /19/.

![Figure 7: Different manipulating tools, which can be assembled to the microrobot arm. A) Microelectrodes array, B) micro-coils for magnetic detection; C) micro-machined cantilever tips.](image)

5. BIOLOGICAL MANIPULATION EXPERIMENTS

Cells used in the experiments were those of Yeast (Saccharomyces Cervisiae) (CECT 1170), grown at 26ºC in bi-distilled water with a dextrosated sabouraud agar (138) and glucose (10%) and harvested in the stationary phase after two days. Also Escherichia Coli was used, grown at 37º in LB agar medium for one day. After the process, both types of cells are diluted in distilled water to achieve the desired concentrations.

The measurements, were made using castellated interdigitated microelectrode structures. They were fabricated on a planar silicon substrate using photolithography in the case of the 500 Amstrongs gold on 1000 Amstrongs titanium and a lift-off process, in the case of 1500 Amstrongs platinum on 500 Amstrongs titanium.
Dielectrophoretic sorting is a consequence of the different electrical properties (conductivity and permittivity) existing between a particle and its surrounding medium. Let consider a dielectric spherical particle of complex permittivity:

\[ \varepsilon_p^* = \varepsilon_p - j\frac{\sigma_p}{\omega} \]

when is immersed in a dielectric fluid medium of permittivity:

\[ \varepsilon_m^* = \varepsilon_m - j\frac{\sigma_m}{\omega} \]

and subjected to uniform z-directed AC electric field of magnitude \( E_0 \) at radian frequency \( \omega \). The solution for the potential outside the particle takes the form:

\[ \Phi(r, \theta) = -E_0 r \cos \theta + \frac{P_{eff} \cos \theta}{4\pi\varepsilon_0 r^2}, r > R \]

with an effective moment:

\[ P_{eff} = 4\pi\varepsilon_m a^3 \left( \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p + 2\varepsilon_m} \right) E_0 \]

Due to this effective moment, a net dielectrophoretic force acts over the particle:

\[ \vec{F}_{DEP}(\omega) = 2\pi\varepsilon_m a^3 \text{Re} \left( \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p + 2\varepsilon_m} \right) \left| \mathbf{E}_{rms} \right| \]

Being \( \mathbf{E}_{rms} \) the rms value of the field, and \( a \), the radius of the particle.

The principle of the dielectrophoretic sorting is the fact that particles with different electrical properties can have opposite kinetic behaviour under an applied electric field. Depending on the frequency chosen it is possible to attract one type of particle to the electrode edges (e.g. castellated electrodes) where a non-homogeneous electric field is applied and repel another.

Figure 8 shows cellular separation experiments using a solution with yeast cells (Saccharomyces Cervisiae) and bacteria Escherichia Coli in concentrations of \( 10^7 \text{ ml}^{-1} \) and \( 5\cdot10^7 \text{ ml}^{-1} \) respectively.

An AC voltage of 5 volts in amplitude has been applied to the castellated electrodes (squares dimensions are 70 \( \mu \text{m} \) long). The frequency of the voltage can be swept from 2 MHz to 20 MHz.

Particle movement and positioning were observed using Karl Zeiss stereomicroscope with a Sony CCD color video camera (SSC-C370P). They were recorded in a computer by means of an image acquisition card and with the help of a image processing software a frame-by-frame study could be performed.

The image on the left has been taken when the AC frequency is around 2 MHz. In this conditions both E-coli and Saccharomyces undergo a p-DEP condition so that the high field densities of the electrodes edges attract all the cells in the solution. But the dielectric behaviour with the frequency of the samples is not the same. So there is a range of frequencies where one of the cellular types is under p-DEP and the other one is under n-DEP. This situation is reflected in the right side image of Figure 2. For an excitation frequency around 20 MHz the E-coli cells exhibit a p-DEP behaviour but the Saccharomyces are under n-DEP. That is why the yeast cells are moved away from the edges of the microelectrodes.
The development of bio-handling devices based on dielectrophoresis require careful elucidation of the double layer dynamics, formed when an oscillating voltage is applied to the electrode surface, and in the response with the field inhomogeneities of induced dipoles over the particles. Several authors [25,18] since the first DEP experiments using microelectrodes have reported anomalous low-frequency particle attraction and deposition over the electrodes, and recently has been partially attributed to the electroosmotic fluid flow movement /19,20/

6. CONCLUSIONS

A new approach to build piezoelectric-based miniature robots with sizes around 1cm³ has been developed as a first approach for biological applications. The system is based on the concept of smart piezoactuator unit and can be adapted to include different arms capabilities for cell handling and manipulation as microelectrodes to produce non-homogenous electric fields, micro-coils to detect magnetic attached spheres and cantilevers tips for nano range probe applications.

Experimental results showing cells sorting by means of microelectrode array and dielectrophoretic forces phenomena have been presented. They confirm the capabilities of these microrobots which include enough degrees of freedom to hold the manipulating tools, according to the specific robot task.

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