MeDICS
Microelectronic Devices for Individual Cell Sorting

MeDICS
IST–2001-32437
Microelectronic Devices
for Individual Cell Sorting

Final Report

Covering period 1 November 2001 to 31 July 2004

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Project Co-ordinator:	Silicon Biosystems
Partners:	Unibo, CEA, Inserm

Information Society

Project funded by the European Community under the "Information Society Technologies" Programme (1998-2002).

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Executive Summary

The MeDICS project has blazed a trail in cell-biology lab-on-a-chip holding the promise to deliver biologists unprecedented capabilities in term of miniaturization, selectivity and programmability in a highly integrated device.
The goal of this project has been twofold:

1. To study and implement a microelectronic circuit that is able to manipulate single cells using the superficial electric field generated by a silicon chip. The device is thus based on a working concept that does not require mechanical fluidic engines such as pumps, valves, hydrodynamic engines or flow cytometry.
2. To investigate its impact on existing biological cell analysis methodologies by defining application protocols, comparing device performance with that of existing assay procedures on a significant biological cell model.

The project has achieved important results in both the technological and biological application perspectives, with a number of "firsts", proof-of-concept demonstrations showing the potential of the technology:

- Design fabrication and microfluidic packaging of the **first microelectronic chip prototype, with integrated actuation and sensing**. The chip integrates more than 100,000 individually addressable electrodes (20μm x 20μm) which enable the creation of more than 12,000 DEP cages in a tiny drop of sample (4ul volume). Moreover, each electrode has an associated sensor (either optical or capacitive) to detect particle presence (feature not considered in the original work-plan, only dealing with manipulation).
- Manipulation of **individual live cells** in the silicon chip**: immortalized cell lines as well as primary cells**. Maniuplation of **clusters of yeasts, RBCs and microbeads**.
- On-chip **Sorting** of a fluorescently labelled subpopulation of lymphocytes from a white peripheral blood cell sample.
- **Flow-less, label-free separation** based on cell physical properties. (red blood cells from K562 cells, beads of different sizes).
- **Particle detection with on-chip optical sensors**: individual 10 to 50μm polystyrene beads, and clusters of RBCs or 3μm polystyrene beads.
- **Particle detection with on chip capacitive sensors**: demonstrated with 50um and 10um polystyrene beads, cluster of yeasts (Yarrowia lipolytica), and K562 cells

Results from the project have been disseminated in top level international conferences (more than 20 conference presentations, 5 journal papers). The remarkable interest from the both the scientific and business community is demonstrated also by the awards won by project participants, due to the outstanding innovations stemming from the project and the commercial potential, such as:

- 2003 IEEE International Solid State Circuit Conference – *Jan Van Vessem Award for Outstanding European Paper*, S. Francisco, CA (Silicon Biosystems and ARCES)
- Runner-up in Life-science category at Science2Market competition for *Most Valuable Proposition*, Pan-european contest on technologies with greatest commercial potential, London, UK, (Silicon Biosystems)
- *Best poster contribution Award* - Congresso Nazionale su Sensori e Microsistemi – Bologna, Italy (Silicon Biosystems, ARCES)

The consortium composition and roles have been as follows:
Silicon Biosystems (I) – Coordination, System level design, silicon design and preliminary testing, optical and capacitive sensing validation, label-free separation.
ARCES (I) – Physical simulation and modelling, silicon design and preliminary testing, optical and capacitive sensing validation, label-free separation.
CEA-DSV (F) - Biological preliminary testing, fluorescence-based, and label-free separation, final validation
CEA-LETI (F) – Electrical packaging, development of Fluidic Packaging techniques, chip surface modifications.
INSERM (F) –Cell models and fluorescent/bead tagging techniques, bead-based separation, final biological validation.

Project objectives

The goals of this project has been twofold:

1. To study and implement a microelectronic circuit that is able to manipulate single cells using the superficial electric field generated by a silicon chip. The device is thus based on a working concept that does not require mechanical fluidic engines such as pumps, valves, hydrodynamic engines or flow cytometry.
2. To investigate its impact on existing biological cell analysis methodologies by defining application protocols, comparing device performance with that of existing assay procedures on a significant biological cell model.

Methodologies

The methodology employed by the MeDICS project is characterized by the use of a microelectronic substrate (based on standard CMOS technology), which is unique in the landscape of lab-on-a-chip for cell-analysis.

The are several advantages related to the use of a microelectronic substrate:

Massive parallelism – More than 100,000 electrodes have been integrated on the chip, since row and column decoders and local memories allow one to memorize the electrode array configuration.

Sensors integration – optical sensors can be directly integrated in the CMOS chip. enabling the detection of individual particles or clusters. Impedance sensing could also be integrated thanks to the availability of pixel-level addressing and amplification of the weak-signals related to the particle presence.

Low-cost manufacturing – due to the batch fabrication using silicon planar technology, chip cost could be driven down to about 1-10 euro, depending on production volume.

Structured design methodology – The CAD tools developed by the microelectronic industry can be reused for the design of the chip, although they must be complemented with physical level simulations.

As detailed in what follows, lab-on-a-chip for cell handling is confirmed to be an emerging technology, and despite the remarkable achievements of the most advanced companies working in this sector, the approach pursued in the MeDICS project significantly advances the state-of-the-art, as far as programmability, parallelism, and integration are concerned.

World-wide 'state-of-the-art' update

Evotec technologies (www.evotech-technologies.com) based in Germany, has begun commercialisation of lab-on-a-chip devices for dielectrophoretic cell manipulation, based on the work of professor G. Fuhr (Fraunhofer Institut fuer Biomedizinische Technik, and Humboldt University Berlin). Cytocon™ and Cytoman™ (implementing a single DEP cage, plus dielectrophoretic filters) products afford particle separation within a flow-through system, or particle immobilization (only one DEP cage is available). The declared unit price for the Cytocon™ Sorter 4 chips is 1,300€ (price for the driving instrument –Cytocon™ 300– is not known).

> *Advantages* (with respect to MeDICS device): (i) the chips use a glass substrate, which allow one to easily use conventional inverted microscopes (ii) They are already on the market.
>
> *Disadvantages*: (i) the Cytocon™ implements just one DEP cage, as opposed as more than 10,000 cages of the first MeDICS prototype (ii) the cage cannot be moved, so particle motion in/out of the cage requires controlled fluid flows, while each individual DEP cage can be controlled independently in the MeDICS device (iii) a passive substrate makes very difficult to integrate sensors on the chip (as opposed to the microelectronic substrate employed by the MeDICS chip).

Genoptix (www.genoptix.com), a California based company, is developing a technology based on laser tweezers for cell manipulation. The technology, called Optophoresis™ uses biocompatible infrared lasers to trap cells and move them by shifting the laser beam. The principle is conceptually similar to the moving dielectrophoretic cages concept patented by Silicon Biosystems: a fieldtrap is created, then particle can be moved by shifting the field trap. At Chips-to-Hits 2002 the company demonstrated experimental results for the label-free separation of cell populations in a flow-through device with microfabricated channels, where the deflection is achieved by the laser beam.

> *Advantages* (with respect to MeDICS device): (i) the chips use a glass substrate, which allow one to easily use conventional inverted microscopes (ii) they do not need to pattern electrodes in the microchamber (iii) the approach is claimed to be compatible with 96-well microtiter plates.
>
> *Disadvantages*: (i) Genoptix technology afford individual particle manipulation, but only one cell can be individually handled at a time, as opposed as more than 10,000 cages of the first MeDICS prototype (ii) so far their demonstrated particle motion relying on controlled fluid flows, while each individual DEP cage can be

controlled independently in the MeDICS device (iii) a passive substrate makes very difficult to integrate sensors on the chip (as opposed to the microelectronic substrate employed by the MeDICS chip)

Fluidigm (www.fluidigm.com), a spin-off of Caltech, California, demonstrated[1] microfluidic chips based on *soft-lithography*. The microfabricated silicone-elastomer devices integrating 2056 pressure controlled valves, implementing 256 individually addressable sub-nanoliter reaction chambers. The fluid flow into/out of the chambers is achieved by pressure driven flow through multiplexed I/O channels. They showed experimental results of (among the other things) assay performed on cells segregated in the microchambers.

Advantages (with respect to MeDICS device): (i) the chips use a transparent substrate, which allow one to easily use conventional transmission microscopes (ii) each microchamber may be filled with different reagents, thus making easier to deliver different compounds/dosages to the cells under test.

Disadvantages: (i) Fluidigm technology afford individual particle manipulation, but cell manipulation does not prevent contacts with the device surfaces, resulting in a device which is prone to clogging of channels and sticking of cells to the microchamber surfaces. (ii) The number of chambers (256) is considerably lower as compared to the 10,000 cages of the first MeDICS prototype (iii) a passive substrate makes very difficult to integrate sensors on the chip (as opposed to the microelectronic substrate employed by the MeDICS chip)

Adeptas (www.adeptas.com) based in Texas, US, is a spinoff from the University of Texas, led by P. Gascoyne, one of the pioneers in Dielectrophoresis. The website shows some pictures of a product called *DEP Counter* which aims to be an alternative to Coulter Counters, claiming higher cell counting discrimination method and an order of magnitude reduction in cost. *DEP Counters* will also enable cell viability testing and apoptosis monitoring in tissue culture labs and cell production facilities.

Fig. 1 Adeptas products

No data sheet nor costs estimates are available about this product. Individual manipulation, as afforded by the MeDICS prototype, is not possible. The adeptas technology could be suitable for label-free separation but does not allow precise control of cell positions.

Immunicon (www.immunicon.com) based in Pennsylvania, US, has developed a diagnostic platform based on immuno-magnetic selection and fluorescent characterization of rare cells in blood. The Company's principal focus is cell-based and molecular diagnostic products for the diagnosis, staging and monitoring of cancer. It has 24 issued and 22 pending US patents. The technology is based on magnetic particles and CD technology.

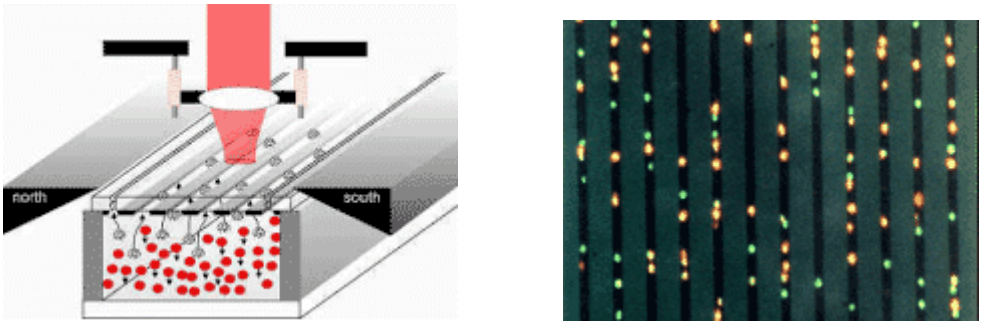


Fig. 2: left: Sketch of Immunicon CellTrack™ system. Actuation for focusing cells is achieved by magnetic particles. Detection is achieved through external microscopes with fluorescence – right: images of cells through nickel tracks of CD

Individual manipulation, as afforded by the MeDICS prototype is not possible. As of today it does not seem easy for them to recover the rare cells of interest (although they claim prototype for this are being investigated).

[1] T. Thorsen, S. J. Maerkl, S. R. Quake, "Microfluidic Large-Scale Integration", *Science*, vol 298 pp 580-584, 18 October 2002

Project results and achievements

Assesment of project results and achievements – Project's Achievements Fiche

Questions about project's outcomes	Number	Comments
1. Scientific and technological achievements of the project (and why are they so ?)		
Question 1.1. Which is the 'Breakthrough' or 'real' innovation achieved in the considered period	N/A	**What:** The chip demonstrated the possibility to manipulate individually, under software control, tens of thousands of individual cells, and to be able to selectively move selected cells (fluorescently labeled). **Why:** This capabilities are the cornerstone of a wealth of analytical procedures which will be enabled by this technology. **What:** First demonstration of individual detection of cells by an array of capacitive or optical integrated sensors. **Why:** This enriches the functionalities of the chip, opening the possibility to implement portable devices for cell counting and complex analytical protocols. In Addition to optical sensing, capacitive sensing offers a new method of detection, increasing the possibility to discriminate different populations.
2. Impact on Science and Technology: Scientific Publications in scientific magazines		
Question 2.1. Scientific or technical publications on reviewed journals and conferences	19 conf. 5 journals	See section Deliverables and References for a complete list.
Question 2.2. Scientific or technical publications on non-reviewed journals and conferences	2 conf 1 journal	**A Programmable Lab-on-a-Chip for Individual Cell-Biology** Mainstreaming Microfluidics 2003, May 15-16 2003, Boston, MA, (USA) Partners: Silicon Biosystems **DEPArray™: A versatile cell-based active array** Eurobiochips 2003, May 21st 2003, London, UK Partners: Silicon Biosystems **There is No Other Way to Do it: DEPArray™ Lab-on-a-chip Platform for Individual Cell-Biology** World Market Research Centre - Business Briefing: Future Drug Discovery 2003 Partners: Silicon Biosystems
Question 2.3.	1	**Biochips and Cell Manipulation: The MeDICS Project** MINATEC 2003, 22-26th Sep. 2003, Grenoble (F)

Invited papers published in scientific or technical journal or conference.		Partners: all
3. Impact on Innovation and Micro-economy		
A – Patents		
Question 3.1. Patents filed and pending	0	When and in which country(ies): Brief explanation of the field covered by the patent:
Question 3.2. Patents awarded	0	The technology developed within this project has been already patented by the industrial partner Silicon Biosystems, in 1999. EPO has given his OK to issueing of the patent which will be awarded soon. Two further patents on applications where filed before the project start.
Question 3.3. Patents sold	0	When and in which country(ies): Brief explanation of the field covered by the patent* (if different from above):
Questions about project's outcomes	**Number**	**Comments or suggestions for further investigation**
B - Start-ups		
Question 3.4. Creation of start-up	No	Silicon Biosystems, is indeed a start-up but it was founded before project start. Nevertheless we can well say that this project had a tremendous impact in propelling the development of Silicon Biosystems patented technology
Question 3.5. Creation of new department of research (ie: organisational change)	No	Name of department and institution/company:
C – Technology transfer of project's results		
Question 3.6. Collaboration/ partnership with a company ?		Which partner : Which company : What kind of collaboration ?
4. Other effects		
A - Participation to Conferences/Symposium/Workshops or other dissemination events		
Question 4.1.		

Active participation[2] to Conferences in EU Member states, Candidate countries / NAS. (specify if one partner or "collaborative" between partners)		Names/ Dates/ Subject area / Country:
Question 4.2. Active participation to Conferences outside the above countries (specify if one partner or "collaborative" between partners)		Names/ Dates/ Subject area / Country:
B – Training effect		
Question 4.3. Number of PhD students hired for project's completion	3	In what field: 3 Electrical Engineering
Questions about project's outcomes	**Number**	**Comments or suggestions for further investigation**
C - Public Visibility		
Question 4.4. Media appearances and general publications (articles, press releases, etc.)	5	Please refer to section **Errore. Il risultato non è valido per una tabella.** for details
Question 4.5. Web-pages created or other web-site links related to the project	3	www.siliconbiosystems.com/MeDICS www-dsv.cea.fr/content/cea_eng/d_dep/d_drdc/d_biopuces/MeDICS.htm www.arces.unibo.it/wp/wp1/ldr2/prog11/prog11p.htm
Question 4.6. Video produced or other dissemination material		References: (Please attach relevant material)
Question 4.7. Key pictures of results		References: (Please attach relevant material .jpeg or .gif)

[2] 'Active Participation' in the means of organising a workshop / session / stand / exhibition directly related to the project (apart from events presented in section 2).

D - Spill-over effects		
Question 4.8. Any spill-over to national programs	Yes	If YES, which national programme(s): Fondo Italiano per la Ricerca di Base (FIRB) - Project Funded by Italian Ministry of Research & University (MIUR) – worth 2.8M€ co-financed by MIUR for 1.7M€ Progetti di Rilevante Interesse Nazionale PRIN - Project Funded by Italian Ministry of Res. & Univ.
Question 4.9. Any spill-over to another part of EU IST Programme	Yes	If YES, which IST programme(s): Improving Human Resource and mobility. With Project MICS, 2 post-graduate and 2 post-doc personyears Marie Curie Industry Host Fellowships were awarded to Silicon Biosystems for training of young researchers in the exploitation of the technology platform set-up in the MeDICS project. EMERGE program of IHP : project μF² in collaboration with IMM (Institut of Mikrotechnik of Mayence) Comparison of mould fabrication techniques for hot embossing replication of micro-fluidic devices based on Medics design.
Question 4.10. Are other team(s) involved in the same type of research as the one in your project ?	No	If YES, which organisation(s):

Deliverables and References

The following table summarizes the project deliverables. The deliverables D2-2 and D3-2 (shaded) were cancelled since there was no need to fabricate a second silicon chip. The major deliverables (yellow lines) are detailed below.

Deliverables list

Deliverable No[3]	Deliverable name	WP no.	Lead participant	Estimated person-month	Del. type	Security	Delivery (proj-month) [4]
D1-1	Simulation tool	1	UniBO	8	Demonstrator	Restricted	T0+3
D1-2	Scaled-up device + board + software	1	UniBO	9	Demonstrator	Restricted	T0+5
D2-1	Design review report, chip no.1	2	UniBO	7	Report	Internal	T0+6
D2-2	Design review report, chip no.2	2	UniBO	7	Report	Internal	T0+25
D3-1	Silicon chip no.1	3	SiBio	30	Prototype	Internal	T0+9
D3-2	Silicon chip no.2	3	SiBio	29	Prototype	Internal	T0+25
D4-1	Package prototype A2	4	CEA/LETI	18	Prototype	Internal	T0+10
D4-2	Report on the material and technology validation	4	CEA/LETI	2	Report	Internal	T0+23
D4-3	Package prototype B2	4	CEA/LETI	13	Prototype	Internal	T0+28
D4-4	Report on the estimated reproducibility of the technology process	4	CEA/LETI	2	Report	Internal	T0+33
D5-1	Report on the cell models, beads and labeling tools	5	INSERM	9	Report	Internal	T0+10
D5-2	Report on evaluation of the device performance	5	INSERM	9	Report	Internal	T0+33
D6-1	Report on validation of the cage concept	6	CEA/DSV	8	Report	Internal	T0+23
D6-2	Report on validation of the cage concept	6	CEA/DSV	7	Report	Internal	T0+33

[3] Deliverable numbers in order of delivery dates: D1 – Dn
[4] Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.

D6-3	Report on the results of the device with a full significant protocol	6	CEA/DSV	10	Report	Internal	T0+33
D7-1	Final review report	7	SiBio	1	Report	Internal	T0+33
D7-2	Technological Implementation Plan	7	SiBio	2	Report	Public/ Internal	T0+33

Deliverable Summary Sheet – Unibo – D1.2, T0+5, Scaled up device+board+software

Scaled-up devices and testing board with graphical-user-interface programming software and a user manual, for dielectrophoretic characterization of cell populations.

In order to characterize the dielectrophoretic spectra of populations, a 1-D scaled-up device implemented with printed circuit board (PCB) technology was fabricated. The new system delivered to biological partners, consists of a small PCB board holding the biological solution and electrically connected with a motherboard used to generate the electrical signals. The motherboard is connected to a PC which runs a software (DEPscanner) with graphical user interface. With DEPScanner the experimental protocol can be reconfigured by the biologists to perform various different experiments, including dielectrophoretic spectra characterization, cell separation, cell concentration.

A three-day training course, which was attended by representatives of CEA-DSV and INSERM, was organized in Bologna and the instrument was delivered (March=T0+5).

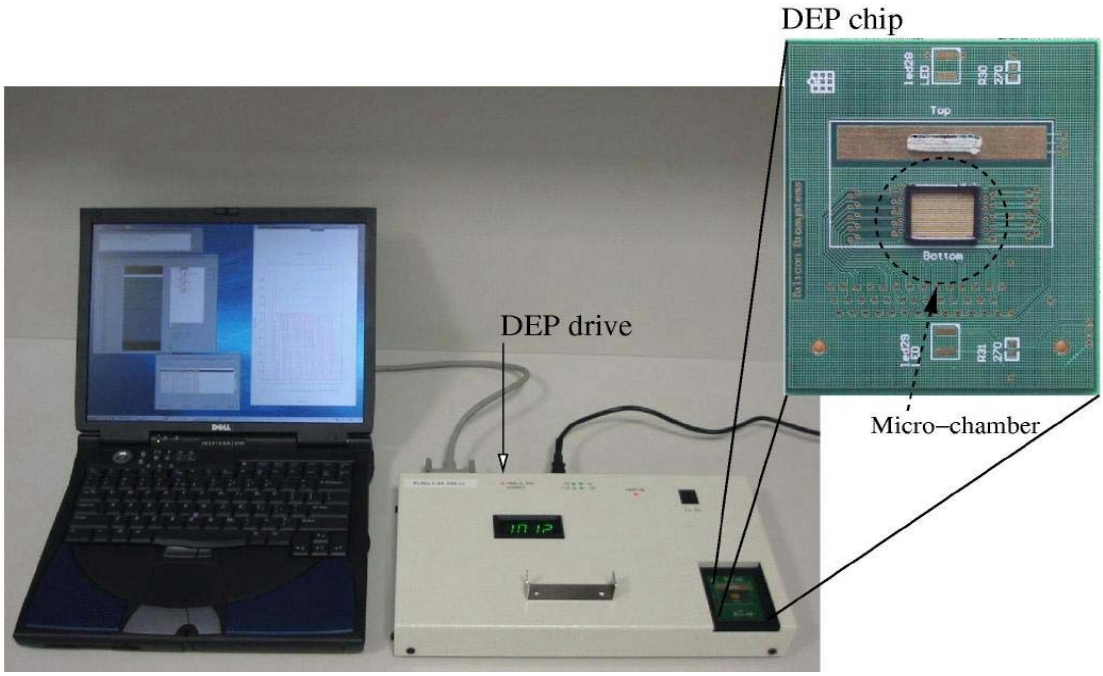


Photo of the Instrument with software and PCB device.

The scaled-up device has been used for:

- Evaluating the simulation tool (developed in D1-1);
- Performing preliminary dielectrophoretic spectra characterization of cells and micro-beads (D5-1).

Partners owning: Unibo

Partners contributed: Silicon Biosystems

Made available to: CEA, INSERM

Deliverable Summary Sheet – SiBIO – D3.1, T0+9, Silicon Chip No. 1

Subitems (delivery time): Chips (T0+6); Electrical testing (T0+8), Chip control board + SW: (T0+13)

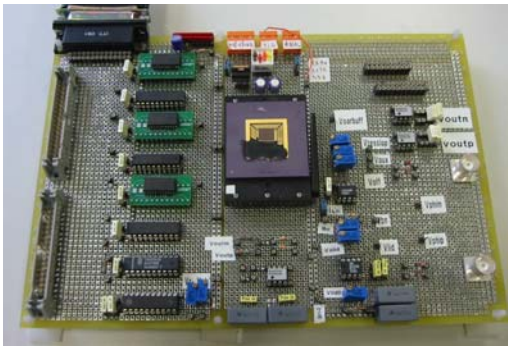
Short Description: **Silicon Chip no. 1**

Silicon chips were fabricated through Europractice by AMS. A CMOS 0.35um technology multi-project run was used. 10 chips in electronic packaging (PGA144) were used for electrical and *early-testing* of silicon basic functionality. 153 naked dies were delivered to CEA-LETI for microfluidic packaging.

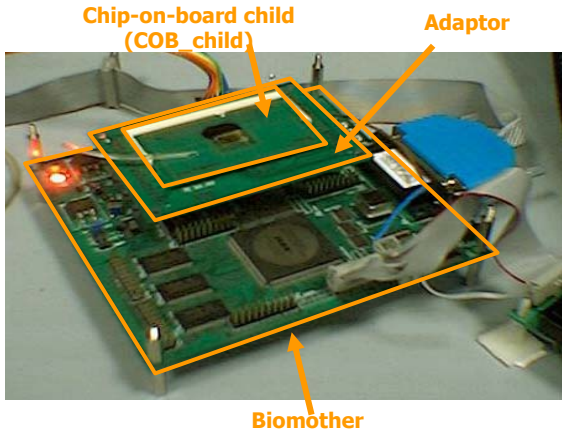
An *early-test* board (wire-wrapped) was designed to accomplish preliminary electrical and functionality tests compatible with the PGA144 chip package. This allowed us to verify that: i) the on chip digital circuits (addressing, memories) are ok ii) on chip analog circuits (bias, readout amplifiers, pixel-level analog multiplexers, and optical sesnors) behave as expected (iii) polystyrene microbeads can be moved and detected.

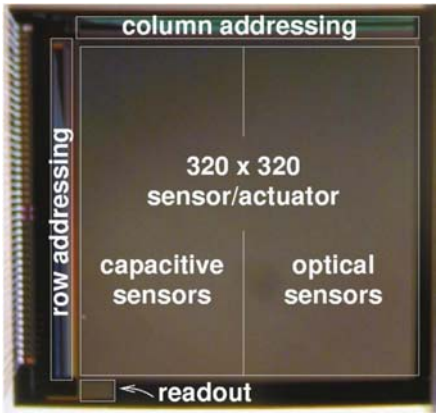
A full-feature chip control board (codenamed *Bio-mother*) was also designed for delivery to biological partners. This board include circuits to generate the actuation stimuli (digital frequency synthesis, amplitude control), and to read out the sensors data (programmable waveform generators, A/D conversion). The board is managed by a XiRISC microprocessor implemented on an FPGA which integrates all the main digital functions (waveform generators, parallel port control, instruction RAM). The *Bio-mother* has been designed to be compatible also with the second MeDICS prototype, and include features such as compatibility with different chip supply voltages, extended addressing space for larger arrays, peltier-effect heat-sink/source for thermal control of the biochip.

Three ancillary printed circuit board devices were designed: *Bio-Adaptor*, *COB-Child* and *PGA-Child*. The *Bio-Adaptor* plugs into the mother board and houses a SIMM connector into which *COB-Child* or *PGA-Child* can be plugged. The *COB-Child* was designed for chip-on-board mounting of the silicon chips. About 300 of these boards were delivered to CEA-LETI for packaging of the devices and glue tests (see deliverable D4-1). The *PGA-Child* adaptor was designed to connect the devices in electronic package PGA144 to the *Bio-Mother,* for testing & debugging of *Bio-mother* itself.

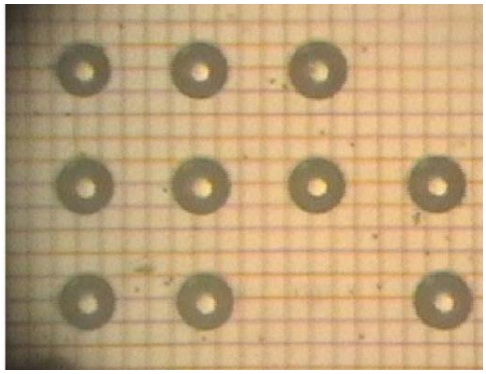


Early-test board

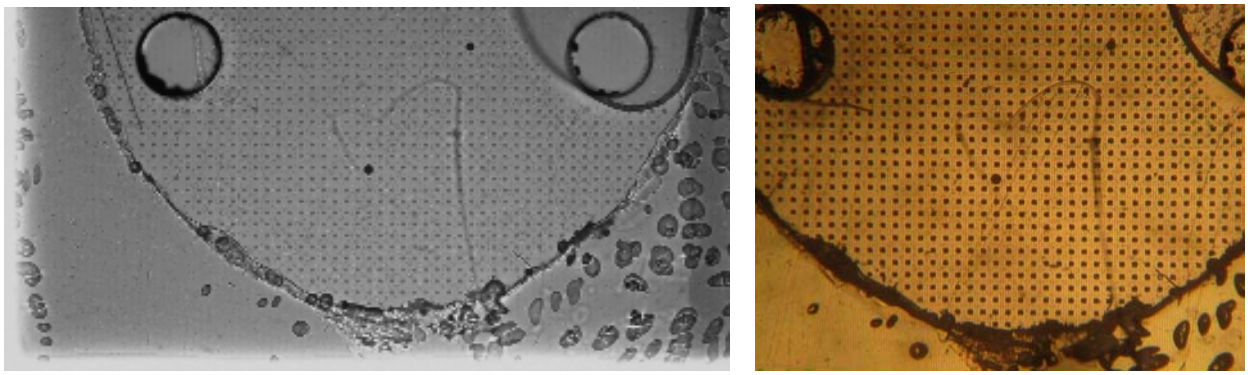




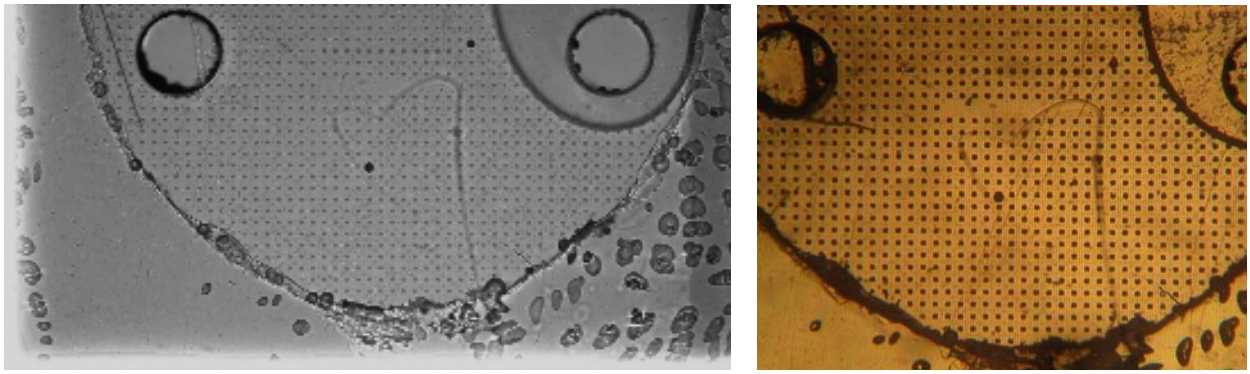
Chip photo



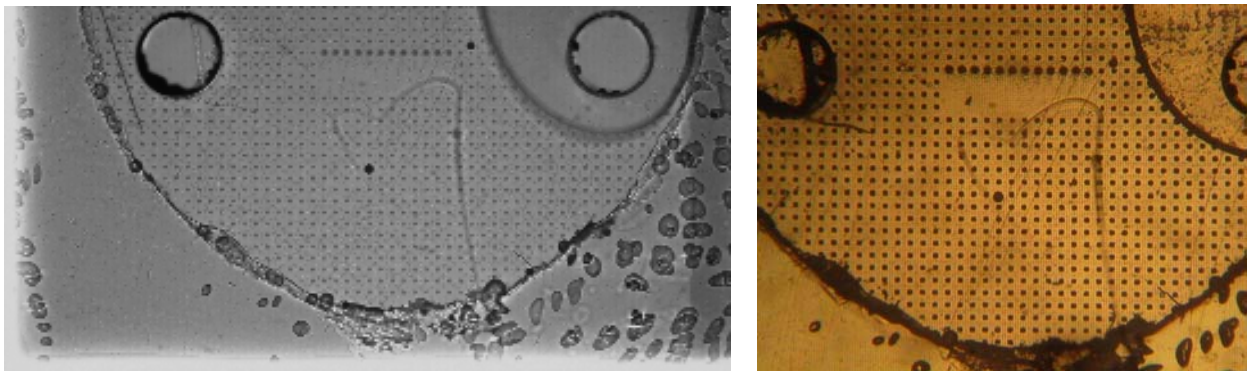
50um beads arrayed by DEP cages



(t0)

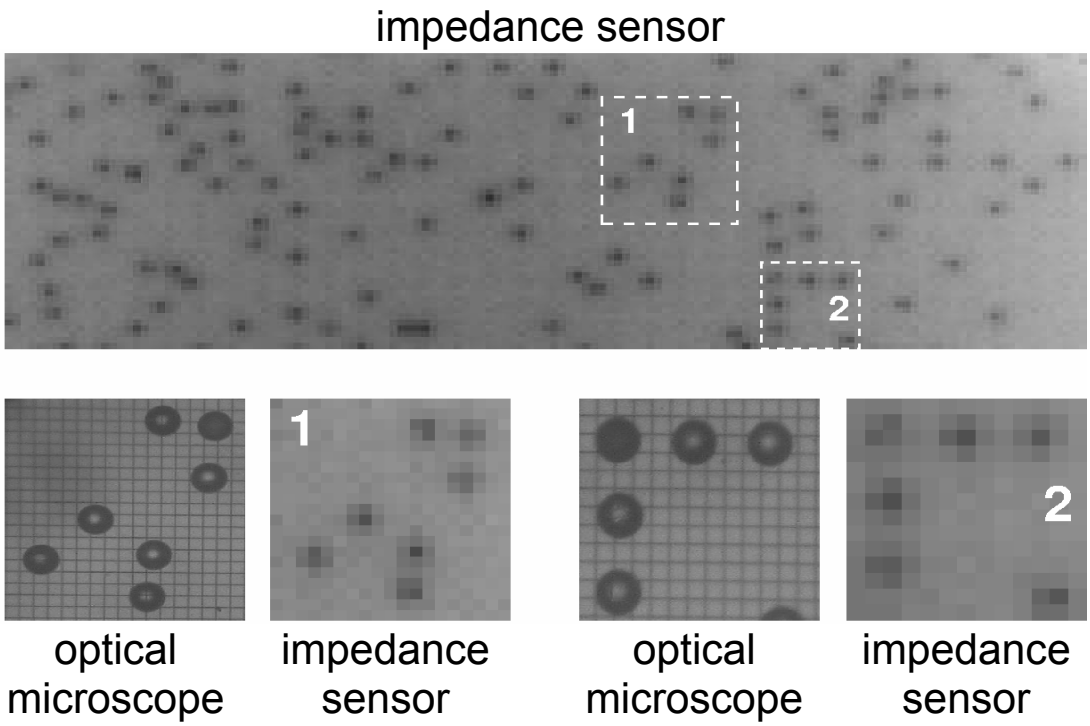


(t1)

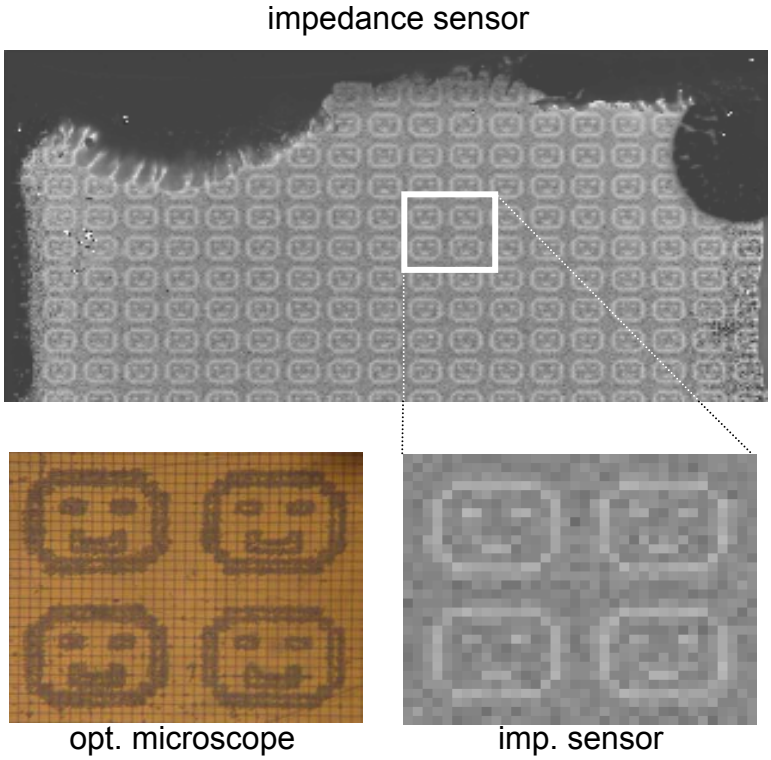


(t2)

Manipulation (cage2gap3 pattern) and optical detection of RBCs, in Mannitol 280mM + 200uM EDTA. On the left, embedded optical sensor scanning. On the right, optical microscope image. At (t0) we have clusters of RBCs with roughly the same number of cells all over the chip. Only the bottom half of the round microchamber (delimited by a pressure sensitive adhesive tape) is associated with optical sensors. The two smaller circles at the top are holes in the cover lid for fluidic input and output (a bubble around the right-side opening is enlarging during the experiment). Over a 60 electrode-wide area (corresponding to 12 cages), four rows of cages are shifted and packed up to merge on a single row. At (t1) the first row has merged with the top one, resulting in a doubling of the density of cells in the clusters, An increase in the darkness of the sensor outputs, can be detected in the top row, while the bottom row is cleared from cells. At (t2) three cages have been merged with the top one. Three rows are cleared, and the top one is giving a stronger signal.



Capacitive sensor image of 50µm polystirene beads



Capacitive sensor image of yeasts (Yarrowia lypolitica)

Partners owning: Silicon Biosystems

Partners contributed: UniBo

Made available to: CEA-LETI, CEA-DSV, INSERM

Deliverable Summary Sheet – CEA/LETI – D4.1, T0+10, Package Prototype A2

Developments:

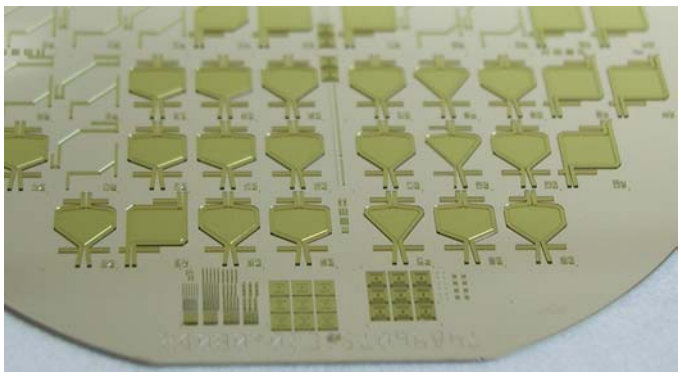
The design of this first prototype include: one chamber, one inlet and one outlet for capillary injection of the sample. At this stage chamber shape are still studied: shape A minimize the fluidic risks and shape N which optimise the useful silicon area.

The technique used to realize these prototypes is the back-up solution listed in the proposal: the fluidic structure is made using SU8 thick lithography on a quartz wafer. A 2 level lithography technique has been developed for this purpose. The conductive transparent layer is made by a ITO layer, a low temperature deposition technique has been developed to be compatible with polymer cap.

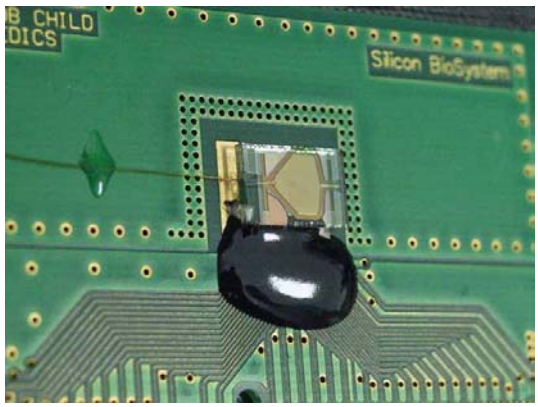
For the assembling, a special bonding technique is used to bond the polymer cap to the silicon chip. This technique allows the deposition of a 1µm thick layer of glue, necessary to obtain a hermetic gasket without filling the thin fluidic chamber and inlets. The packaging step includes the assembling of the microfluidic component on the PCB which was developed using automated equipment. The wire bonding step is realized by a sub-contractor.

All the materials employed for the component as well as each technical step have been validated by biological and fluidic experiments. For this validation more than 20 MeDICS A1 components were assembled on raw silicon and tested from June to October 2002.

Results: Biological test shows a problem of cell adhesion on the ITO layer when the DEP cages are activated. SU8 cap shows a large fluorescence which perturbs the microscope observation of the marked cells.



SU8-on-glass lids, 4" wafer



Silicon chip with microfluidic packaging (plastic lid + capillary) and electrical connections (glob topped) mounted on PCB COB-child

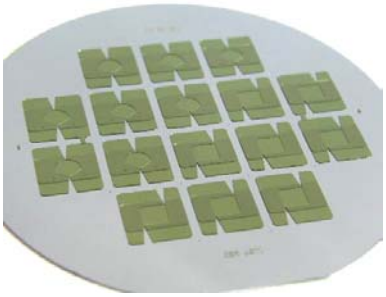
<u>Manufacture and assembly of 60 MedA devices using double layer SU8</u>

Stabilization of different steps of the fabrication process:

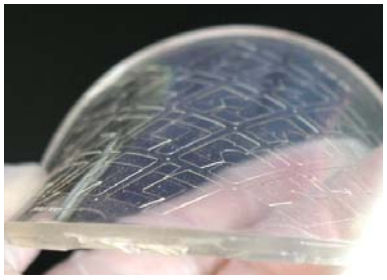
Multiple layer thick photolithography (SU8) process to reach a good surface roughness which is necessary to obtain a good hermetic seal between the Si chip and the fluidic cap.

ITO deposition process was a critical point because the stress of the layer on polymeric structure generates cracks in the ITO in presence of water. Special deposition and substrate preparation processes solve the problem.

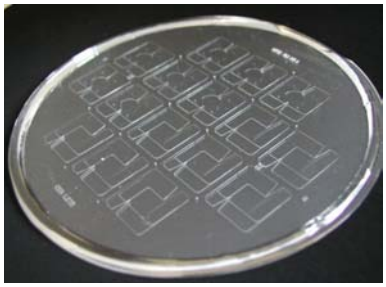
Non fluorescent MedA devices made with Polycarbonate. The goal is to structure the whole µfluidic cap in one replication step to reach low fabrication cost. We developed a technique based on hot embossing with a soft PDMS mould cast on a double layer SU8 master. The advantage of the soft mould is that the hot embossing can be done at a wafer scale by minimizing the demoulding forces.



SU8 Master

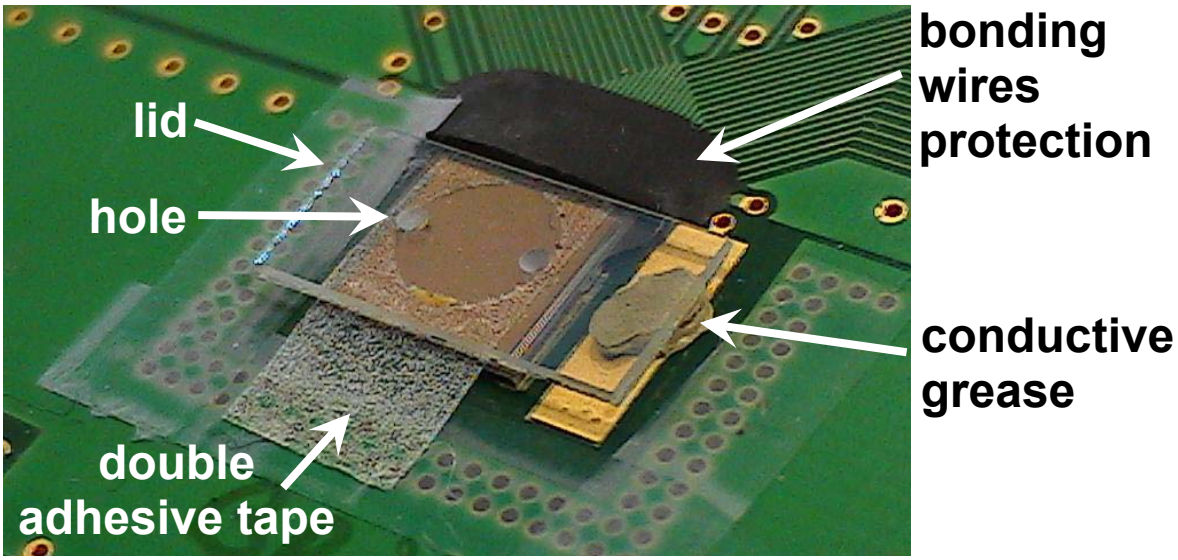


Soft PDMS mould



hot embossed parts in PC

Manufacture of the BRIC device: a completely different device was manufactured using PC or glass substrates which were layered with ITO and pierced to allow loading of biological samples with a pipette. The chamber is defined by a preformed double sided sticky tape.



Deliveries:

60 SU8/ glass MedA

15 Si chips wire-bonded onto PCB for the BRIC devices

30 caps for the BRIC devices

Results: Pipette injection of the sample was preferred to capillary injection, so this changes the conception of the device packaging: the active part (Si chip wire-bonded on PCB) is now re-usable

Only the fluidic cap is disposable and bonding is made using double sided sticky tape.

Partners owning : CEA/LETI

Partners contributed : CEA/DSV, Silicon Biosystems

Made available to : Silicon Biosystems, CEA-DSV, INSERM

Deliverable Summary Sheet – Inserm – D5.1, T0+10, Report, Cell models beads and labelling tools

Short Description:
Various cell models were explored during this first phase of the project. We chose to restrict the first cell models to non adherent type derived from blood line origin: B or T immortalized lymphocytes, hybridomas and red blood cells from fresh blood. These cell lines were chosen keeping in mind final biological & clinical validations in the field of immunology and blood cell line sorting for rare lymphocyte populations.
The first part of the report (contributed by CEA DSV) describes the various tests that were submitted to the cell lines to evaluate the effect of the DEP suspension buffers, of the technological materials and processes on cell viability, recovery and proliferation
The second part (contributed by Inserm) describes the labeling tools that were developed for these cells, the protocols that were optimized to obtain an efficient labeling of a cell line with micron size polycarbonate beads. This bead label will enable the subsequent sorting of the labeled cell population by modifying the dielectric properties of these objects.
The third part of the report (contributed by CEA DSV) is the DEP characterization of these cell lines and beads, using a testing board made available by Silicon Biosystems (the DEP scanner). These results give first indications of the DEP behavior of the above cell lines and labeling tools.

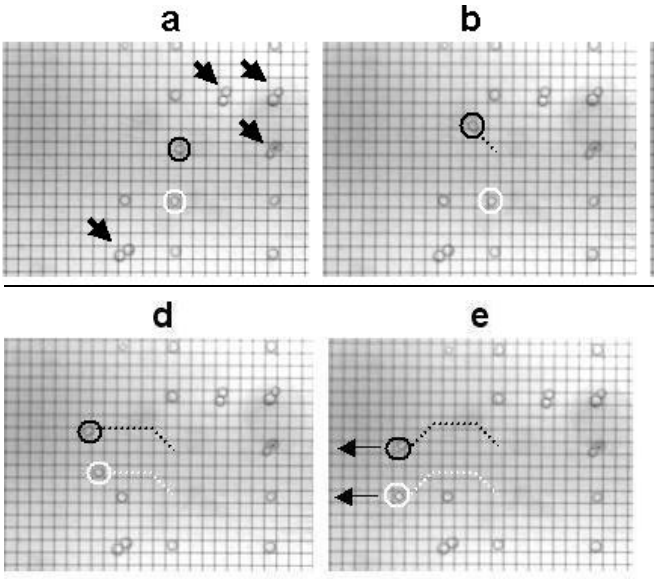
Partners owning:	Inserm
Partners contributed:	CEA-DSV
Made available to:	Silicon Biosystems, Unio

Deliverable Summary Sheet – CEA-DSV– D6.1, T0+23 Report, Biological Validation of the MEDA prototype : Results obtained on levitation, motion and sorting of cells and beads

Short Description:

The report describes all the biological experiments carried out on the first batches of packaged chips. Results are given on the successive batches of ITO deposition, which appeared to be the major parameter in success or failure of our experimental assays.

In particular, the first ITO depositions showed premature aging under experimental conditions. This problem was corrected by our packaging team over successive batches of ITO and the last results which are reported in this document show levitation and motion of various size of beads as well as cultured cell lines.



Sequence of K562 cell manipulation. (a) shows a predefined grid of cages which have trapped cells in 280mM mannitol. Two cells have been chosen and commands are entered to move them 8 steps to the left (b to e) and out of view.

CEA-DSV also worked on software procedures and a user friendly graphical interface and these elements are also presented in the report

The report ends on preliminary results on another type of packaging that was explored in parallel to the SU8 on glass structuration. This packaging presents a number of advantages and will inspire the next packaging for the final year of the project.

These results demonstrate that we have attained our "proof of concept" biological challenge for this silicon chip.

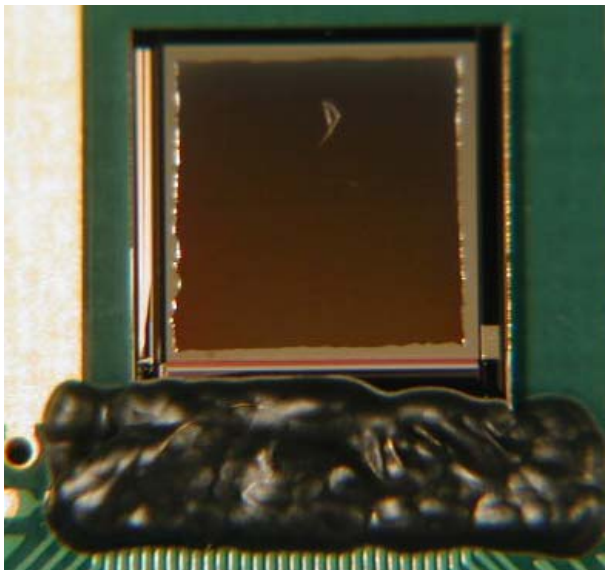
Partners owning:	CEA-DSV
Partners contributed:	
Made available to:	Silicon Biosystems, Unibo, INSERM

Deliverable Summary Sheet – CEA/LETI – D4.3, T0+28, Package prototype B2, double chamber design for cell separation

Developments :

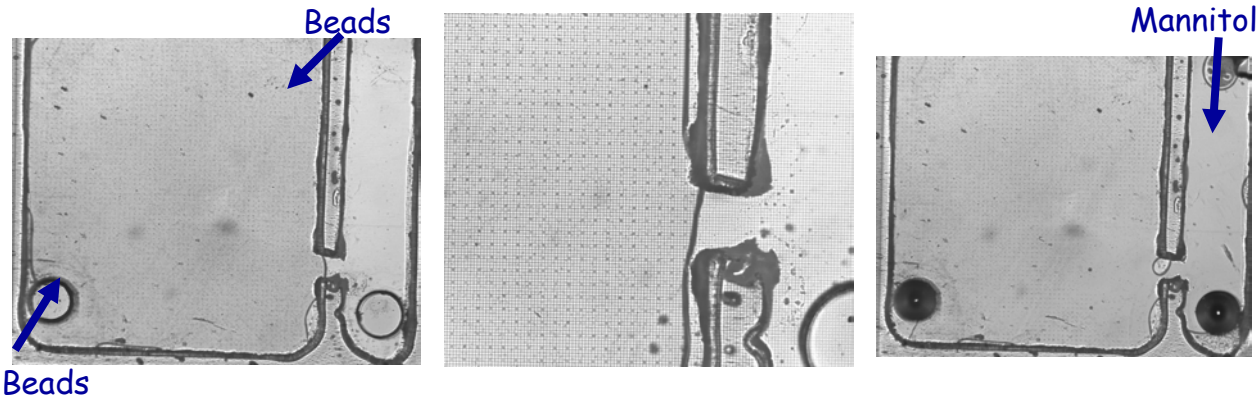
Chip depassivation process

Thininng of the passivation of the CMOS chip was required to lower the voltage drop on the insulator for relatively conductive (>10mS/m) buffers. A Plasma etching process acting on individual dies was developed, and few tens of chips depassivated.



Pictures of plasma-etch depassivated silicon chip, mounted on PCB and glob-topped for wirebond protection.

Fluidic design of the double-chamber double-adhesive tape was carried out, and preformed laser-cut tape (from subcontractor) mounted on glass and PC ITO-coated glasses, and delivered to partners for testing.



Pictures of double-chamber design on the silicon chip, with test beads: sample injection (left), trapping of beads (center), buffer injection in the recovery chamber (right).

Deliveries:

8 MEDT chips (thinned passivation) mounted on PCB

7 MEDU chips (unpassivated) mounted on PCB

More than 70 disposable caps mounted with preformed-tape

Partners owning: CEA/LETI
Partners contributed: CEA/DSV, Silicon Biosystems
Made available to: Silicon Biosystems, CEA-DSV, INSERM

References like articles, conference presentations, seminars and media coverage are listed below

Scientific Conferences

A Microelectronic Approach for Single-Cell Manipulation
M. Tartagni, G. Medoro, N.Manaresi, A. Romani, A. Leopardi, L. Altomare and R. Guerrieri
Congresso Nazionale su Sensori e Microsistemi - 2002, Bologna, I, February, 2002;
Note: The poster presented won the *Best-contribution award*.

Simulation Methodology for Dielectrophoresis in Microelectronic Lab-on-a-chip
A.Leonardi, G. Medoro, N. Manaresi, M. Tartagni and R. Guerrieri;
Modeling and Simulation of Microsystem 2002, Puerto Rico, 22-25 April 2002, pp. 96-99
Note: this work related to the simulation methodology developed and exploited in the Project for simulating particles behaviour in dielectrophoretic fields (see WP1, D1-1)

A Lab-on-a-chip For Cell Detection and Manipulation
G. Medoro, N. Manaresi, A. Leonardi, L. Altomare, M. Tartagni and R. Guerrieri,
IEEE Sensors Conference 2002, Orlando, FL, June, 2002;
Note: work related to the scaled-up PCB prototype delivered to CEA and INSERM (see WP1, D1-2)

A Lab-on-a-chip for Cell Separation Based on the Moving-Cages Approach
G. Medoro, N. Manaresi, M. Tartagni, L. Altomare, A. Leonardi and R. Guerrieri,
Eurosensors XVI, Prague, CZ, September, 2002,
Note: work related to the scaled-up PCB prototype delivered to CEA and INSERM (see WP1, D1-2). An algorithm for label-free separation based on DEP spectral signatures is introduced, validated by experimental results on microbeads-yeast separation.

Microelectronics Meets Biology: Challenges and Opportunities for Functional Integration in Lab-on-a-Chip N. Manaresi, G. Medoro, M. Tartagni, L. Altomare and R. Guerrieri,
European Solid-State Circuits and European Solid-State Devices Research Conferences, ESSCIRC-ESSDERC 2002, Firenze, I, September, 2002,
Note: This invited plenary talk included an overview of microelectronic-based lab-on-a-chip work and presented MeDICS device as the leading-edge technology for cell manipulation.

Software Programmable Labs-on-a-chip for Individual Manipulation and Detection of Thousands of Living-Cells in Microliter Samples
N.Manaresi,
Chips-to-Hits 2002, Philadelphia, PA, October, 2002;
Note: this is the "World's Premier Microtechnology Event for Life Sciences" with attendance form industry and academia. A presentation was given by Silicon Biosystems in the *Emerging technologies start-up showcase* session, centered on the technology developed within MeDICS, with preliminary results on cell manipulation.

Example of hybrid component fabrication for cell manipulation
I. Chartier et al.
New fabrication technologies for polymer microdevices, OMNT-MINATEC Seminar
Grenoble, F, Oct 2002;

MeDICS: A Lab-on-a-chip for Cell Separation Based on Dielectrophoresis
A.Fuchs, D.Freida, I.Chartier, N.Sarrut, C.Villiers, G.Medoro, N.Manaresi, A.Romani, L.Altomare, R.Guerrieri and M.Tartagni
Nanotech 2002, Montreaux, CH, November 2002;
Note: this is the 6th annual European conference on micro & nanoscale technologies for the biosciences. The presentation showed experimental results achieved with the scaled-up PCB prototype and the concept of individual manipulation.

Thick SU8 Lithography for BioMEMS
M. Rabarot et al.

Photonic West, Micromachining & Microfabrication, SPIE conf., San Jose, CA, Jan 2003;

A CMOS Chip for Individual Cell Manipulation and Detection
N. Manaresi, A. Romani, G. Medoro, L. Altomare, A. Leonardi, M. Tartagni and R. Guerrieri
ISSCC 2003, S. Francisco, CA, February 2003;
Note: ISSCC is recognized as the foremost international conference in the field of silicon IC design. Disclosure of the circuit details of the first chip prototype have been given, together with first experimental results. This paper won the Jan Van Vessem Award for Outstanding European Paper (see right).

MeDICS / A CMOS electronic device for individual manipulation of thousands of living cells
A. Fuchs, D.Freida, I.Chartier, N.Sarrut, C.Villiers, G.Medoro, N.Manaresi, A. Romani, L. Altomare, R. Guerrieri, M. Tartagni
Nanobiotechnologies II, Grenoble, April 2003

A Programmable Lab-on-a-Chip for Individual Cell-Biology
N. Manaresi,
Mainstreaming Microfluidics 2003, May 15-16 2003, Boston, MA, (USA)
Note: this is a more business-oriented conference with most presentation from leading companies in the biochip field plus few presentations from academic groups. This conference was organized by the Cambridge Healthtech Institute.

DEPArray™: A versatile cell-based active array
N. Manaresi,
Eurobiochips 2003, May 21st 2003, London, UK
Note: this is a more business-oriented conference with most presentation from leading companies in the field. This conference is the European equivalent of Chips-to-Hits, with same organization from International Business Communications.

A Cell-On-Chip for Individual Cell Manipulation by Dielectrophoresis
Marche PN, Fuchs A, Freida D, Chartier I, Sarrut N, Villiers CL, Derouich-Guergour D, Medoro G, Romani A, Altomare L, Guerrieri R, Tartagni M and Manaresi N.
Micro et NanoBiotechnologies for Medicine and Chirurgy, 26-27th May 2003, Paris,
Organisation « World Academy of Biomedical Technologies » presentation by PN MARCHE

A System-on-a-Programmable-Chip for Real-Time Control of Massively Parallel Arrays of Biosensors and Actuators,
A. Romani, F. Campi, S. Ronconi, G. Medoro, M. Tartagni and N. Manaresi,
The 3rd IEEE International Workshop on System-on-Chip for Real-Time Applications, June, 2003, pp. 236-241
Note: Presentation centered on the digital hardware design.

Biochips and Cell Manipulation: The MeDICS Project
N. Manaresi, A. Fuchs, D. Freida, L. Altomare, C.L. Villiers, G. Medoro, A. Romani,A. Leonardi, I. Chartier, N. Sarrut, M. Tartagni, P.N.Marche, F. Chatelain and R. Guerrieri ,
MINATEC 2003, 22-26th Sep. 2003, Grenoble (F)

A lab-on-chip for cell sorting and manipulation based on dielectrophoresis : effect of beads on cell levitation
D Derouich-Guergour ,CL Villiers, PN Marche, A Fuchs, D Freida, I Chartier, N Sarrut, G Medoro, A Romani, L Altomare, R Guerrieri, M Tartagni & N. Manaresi
MINATEC 2003, 22-26th Sep. 2003, Grenoble (F)

A Microelectronic Chip Opens New Fields in Rare Cell Population Analysis & Individual Cell Biology
A. Fuchs, N. Manaresi, D. Freida, L. Altomare, C.L. Villiers, G. Medoro, A. Romani, I. Chartier, C. Bory, M. Tartagni, P.N.Marche, F. Chatelain and R. Guerrieri .

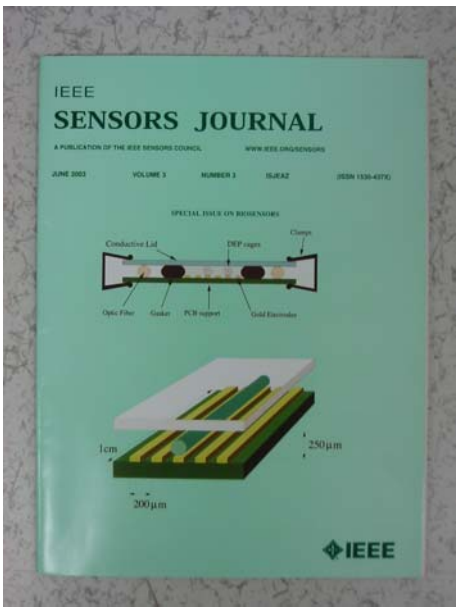
MicroTAS, 6-9 October 2003, Lake Tahoe (USA)
Note: This is the recognized foremost international conference for microsystems in biology and chemistry. Our abstract was selected for an oral presentation given by A Fuchs.

Fabrication of hybrid plastic-silicon microfluidic devices for cell manipulation by dielectrophoresis
I. Chartier, C. Bory, A. Fuchs, D. Freida, N. Manaresi M. Ruty, J. Bablet, K. Gilbert, N. Sarrut, F. Baleras, L. Fulbert. *SPIE 2004*

Capacitive Sensor Array for Localization of Bioparticles in CMOS Lab-on-a-chip
A. Romani, N. Manaresi, L. Marzocchi, G. Medoro, A. Leonardi, L. Altomare, M. Tartagni and R. Guerrieri
ISSCC 2004, S. Francisco, CA, February 2004;
Note: Circuit for capacitive sensing and some experimental results were presented.

Journal Papers

A Lab-on-a-chip for Cell Detection and Manipulation,
G. Medoro, N. Manaresi, A. Leonardi, L. Altomare, M. Tartagni and R. Guerrieri, IEEE Sensors Journal, vol. 3, no. 3, June , 2003, pp. 317-325
Note: This was a special issue on biosensors. A figure from our paper was selected for the cover page (on the right).



Levitation and Movement of Human Tumor Cells Using a Printed Circuit Board Device Based on Software-Controlled Dielectrophoresis
L.Altomare, M.Borgatti, G.Medoro, N.Manaresi, M.Tartagni, R.Guerrieri, R.Gambari
Biotechnology & Bioengineering, 2003 Wiley Periodicals Inc.

Application to Cancer Research of "Lab-on-a-chip" Devices Based on Dielectrophoresis (DEP)
R. Gambari, M. Borgatti, L.Altomare, N.Manaresi, G.Medoro, A. Romani, M.Tartagni, R.Guerrieri,
Technology in Cancer Research & Treatment, Vol. 2, n. 1, February 2003, Adenine Press.
Note: Overview paper featuring also results from PCB devices developed in the MeDICS Project and perspective on the use of the silicon chip.

A CMOS Chip for Individual Cell Manipulation and Detection
N. Manaresi, A. Romani, G. Medoro, L. Altomare, A. Leonardi, M. Tartagni and R. Guerrieri
IEEE Journal of Solid State Circuits, vol 38, n. 12 Dec. 2003.
Note: Follow-up of ISSCC 2003 paper.

Microelectronic Chips for Molecular and Cell Biology
M. Tartagni, A. Fuchs, N. Manaresi, L. Altomare, G. Medoro, R. Guerrieri and R. Thewes
Sensors Update, Wiley
Note: Overview of the field of microelectronic biochips, including results from the MeDICS Project

Media Coverage

French TV News (France2 20heures), A report on CEA-DSV Biopuces lab featured the MeDICS project.
Usine Nouvelle, *La puce qui trie les cellules vivantes,* Anne Pezet
Industrie et Technologies, *Un programme européen pour ðevelopper une puce à cellules*

Following ISSC presentation in S. Francisco we had a remarkable return in media coverage (most of the following have both printed versions and web magazines):

Lab-on-a-chip extended to cell screening
Electronic Engineering Times,
http://www.eetimes.com/at/m/news/OEG20030212S0002
February 12, 2003
Note: this article was published also in EETimes China, and EETimes France

MEMS boost bio-tests
Electronics Weekly,
http://www.electronicsweekly.com/issue/articleview.asp?vpath=/articles/2003/02/12/tech07.htm&mode=archive
February 12, 2003

Researchers push toward true lab-on-a-chip
Silicon Strategies,
February 11, 2003

Salute: ci cureremo così - MICROCHIP Il laboratorio che sta in una mano (Italian)
Panorama
January 9, 2003
Note: this is a widely diffused weekly magazine (probably number 1 or 2 in Italy). The article feature Silicon Biosystems and its technology, developed in MeDICS project.

Un laboratorio in un chip per scoprire le malattie. (Italian)
Il Sole 24 Ore – June 19th 2003
(@lfa – special addendum about IST to the regular newspaper - article related to the Italian tech tour presentation –see below)
Note: *Il Sole 24 ore* is the more authoritative economic newspaper in Italy.

Other (Seminars, market reports etc)

CEA for Europe, Seminar, Brussels, September 10th 2002.

Italian Tech Tour 2003, Venice, June 20 2002.
Silicon Biosystems was accepted for presentation at the Italian Tech Tour, featuring the most innovative and upcoming technology companies in Italy, to an audience of European Venture Capitalists.

Science2Market, London, June 26-27 2002.
Silicon Biosystems ranked second in a European-wide competition among spin-offs from Universities and research centres for "the most valuable proposition" (life-science category), i.e. the business idea/technology with greatest potential to generate the larger return.

Microfluidics: second generation techniques driving commercial applications
Silicon Biosystems is profiled in this Market Report, among the 29 most promising companies in microfluidics for its technology (which is being developed within the MeDICS Project).

Potential Impact of project results

The MeDICS project demonstrated that microelectronics can be leveraged to achieve highly integrated systems with actuation and sensing of individual cells. The approach pursued within the project has a great potential for cell analysis, for research, diagnostic and therapeutic applications.

In the research filed, biologists may be able to study cell-cell interaction with a precise control on the timing, use the chip for isolating fluorescently labelled cells from a small cell-load, or program the system to make complex interactions which might involve, for example, beads, liposomes, cells.

In the diagnostic field, the system could be used to isolate rare-cells from pre-processed samples, in particular it could be the only system affording the possibility to isolate 10-100 cells (too many for manual operation, and more reliable than that) from a population of 10,000-100,000 (too small for Fluorescent Activated Cell Sorters).

Also, due to the integration of sensors, the MeDICS technology could implement a low-cost device for Point-of-Care blood cells analysis, once coupled with some sample collection and preparation stages.

Future Outlook

The MeDICS Project demonstrated that individual cells can be manipulated and detected on a microelectronic chip. The programmability of the chip, one of the advantages of using a microelectronic substrate, opens up various different perspectives for the future exploitation of the research results.

While the biological validation within the project has offered a substantial proof of the viability of this approach and its competitive edge, there are still many opportunities to pursue. The most prominent of these are probably the following:

- Application to the isolation of foetal cells from maternal blood: these rare cells can today be enriched and labelled but a reliable technology for the final isolations of the few cells existing in the original blood sample is required. The MeDICS technology could be the *last-mile* or missing brick of a multi-step protocol with the potential of replacing the more invasive amniocentesis.
- Application to cancer therapy: isolating, with the selectivity afforded by the MeDICS chip, the natural killer cells which are able combat the tumor, could open up the possibility to culture them for reinfusion into the patient.
- Application to point-of-care blood analysis: thanks to the integration of actuation and sensing, the chip could be used for cell-counting at the point-of-care. These would require of course designing a custom, optimized solution to attain the demanding specificity, accuracy and cost constraints of such an application.
- Application to isolation of metastatic epithelial cells in blood for therapeutic research.
- Application to stem cells isolation.

Silicon Biosystems foresee the opportunity to commercialize an instrument based on project results, which could allow researchers to pursue one or more of the above applications. The company is currently seeking funding to carry out the product engineering phase and start commercialization of MeDICS project results.
ARCES (University of Bologna) has launched a 2.8M€, 3-years project, in partnership with the University of Ferrara Biotechnology Centre and University of Perugia, co-financed for 1.7M€ by the Italian Ministry of Research, aimed at the feasibility investigation of some of the potential applications of biochips for cell manipulation and detection based on MeDICS.
CEA intends to continue the development of cell-on-chip technologies based on MeDICS, although possible funding frameworks and continued cooperation with Silicon Biosystems are not yet settled.

Publishable Final Report

Publishable Executive Summary Report

The MeDICS project has blazed a trail in cell-biology lab-on-a-chip holding the promise to deliver biologists unprecedented capabilities in term of miniaturization, selectivity and programmability in a highly integrated device.
The goal of this project has been twofold:

1. To study and implement a microelectronic circuit that is able to manipulate single cells using the superficial electric field generated by a silicon chip. The device is thus based on a working concept that does not require mechanical fluidic engines such as pumps, valves, hydrodynamic engines or flow cytometry.
2. To investigate its impact on existing biological cell analysis methodologies by defining application protocols, comparing device performance with that of existing assay procedures on a significant biological cell model.

The project has achieved important results in both the technological and biological application perspectives, with a number of "firsts", proof-of-concept demonstrations showing the potential of the technology:

- Design fabrication and microfluidic packaging of the **first microelectronic chip prototype, with integrated actuation and sensing**. The chip integrates more than 100,000 individually addressable electrodes (20µm x 20µm) which enable the creation of more than 12,000 DEP cages in a tiny drop of sample (4ul volume). Moreover, each electrode has an associated sensor (either optical or capacitive) to detect particle presence (feature not considered in the original work-plan, only dealing with manipulation).
- Manipulation of **individual live cells** in the silicon chip**: immortalized cell lines as well as primary cells**. Manipulation of **clusters of yeasts, RBCs and microbeads**.
- On-chip **Sorting** of a fluorescently labelled subpopulation of lymphocytes from a white peripheral blood cell sample.
- **Flow-less, label-free separation** based on cell physical properties. (red blood cells from K562 cells, beads of different sizes).
- **Particle detection with on-chip optical sensors**: individual 10 to 50µm polystyrene beads, and clusters of RBCs or 3µm polystyrene beads.
- **Particle detection with on chip capacitive sensors**: demonstrated with 50um and 10um polystyrene beads, cluster of yeasts (Yarrowia lipolytica), and K562 cells

Results from the project have been disseminated in top level international conferences (more than 20 conference presentations, 5 journal papers). The remarkable interest from the both the scientific and business community is demonstrated also by the awards won by project participants, due to the outstanding innovations stemming from the project and the commercial potential, such as:

- 2003 IEEE International Solid State Circuit Conference – *Jan Van Vessem Award for Outstanding European Paper*, S. Francisco, CA (Silicon Biosystems and ARCES)
- Runner-up in Life-science category at Science2Market competition for *Most Valuable Proposition*, Pan-european contest on technologies with greatest commercial potential, London, UK, (Silicon Biosystems)
- *Best poster contribution Award* - Congresso Nazionale su Sensori e Microsistemi – Bologna, Italy (Silicon Biosystems, ARCES)

The consortium composition and roles have been as follows:
Silicon Biosystems (I) – Coordination, System level design, silicon design and preliminary testing, optical and capacitive sensing validation, label-free separation.
ARCES (I) – Physical simulation and modelling, silicon design and preliminary testing, optical and capacitive sensing validation, label-free separation.
CEA-DSV (F) - Biological preliminary testing, fluorescence-based, and label-free separation, final validation
CEA-LETI (F) – Electrical packaging, development of Fluidic Packaging techniques, chip surface modifications.
INSERM (F) –Cell models and fluorescent/bead tagging techniques, bead-based separation, final biological validation.