Summary Report: Mass Produced Optical Diagnostic Labcards Based on Micro and Nano SU8 Layers

Our envisioned diagnostic device will use small raw samples. It will consist of a portable base unit and a disposable Lab on a Card (OptolabCard). The future card platform will contain a chip made by equipment that combines lamination & photolithography of dry films. Sample preparation is being undertaken within the card. The reagents are kept in the LabCard allowing non-expert personnel to use the device. Currently, the developed process based on SU-8 devices has fulfil the expectative and this process will allow us to fabricate very thin devices, and therefore, truly labcards. Two patents have been filled to protect this fabrication knowledge. The successful fabrication process together with preliminary biological miniaturisation good results allow us to feel confident to tackle the rest of the project.

INTRODUCTION

In the late 90´s, the development of chemical and biological sensors played a significant role in improvements of public health, by providing new applications with high sensitivity. **Nevertheless, the commercialisation of such devices has continued to lag behind "research", mainly because this advantage can not currently be offered at low cost.** The following table lists some of the most remarkable approaches:

Manufacturer	Technique	Time	Automa
NEOGEN	Strip	1 day	NO
Merid. Diag.	Strip	1 day	NO
BioMerieux Vitek	Inmunoassay	2 day	YES
Organon Teknika	Inmunomag.	1 day	YES
Dynal Inc	Inmunomag.	1-3 days	NO
Vicam	Inmunomag.	1-3 days	NO
Applied Biosys	Q-PCR	5 hours	YES
Molecular Biosys	PCR	5 hours	YES

List of the available devices in the market ordered by size and time response

It can be seen that neither of them can perform the analytical assay in a quick, simple way within a Lab on a Chip. Up to date, the majority of the existing microsystems have been designed as microscale devices coupled to a macroscale infrastructure. A lack of automation is an outstanding concern due to the cross sensitivity to labour hand since preparative hand steps are needed.

OUR VISION

Our vision of a true Lab on a Card (LabCard) consists of a hand held base unit and a disposable LabCard that will carry out a biology assay automatically, from sample preparation, to detection (i.e. Quantitative Polymerase Chain Reaction qPCR). **The chip, made out of polymer films,** contains all the disposable components (reagents, valves, pumps, beads) and it **is packaged in a Card**, whereas the base unit has all the complex electronics and optics. This approach **will avoid any cross contamination** between measurements and it will allow us to drastically **simplify the chip** components (valves, pumps, reservoirs, sensors, heaters, etc) **since they will need to be used just once.**

CHALLENGES TO BE FACED

Through the first year of the project, many **challenges** are being faced to obtain a commercially viable LabCard: (i) High throughput fabrication process, (ii) few materials involved, (iii) authentic sample preparation, (iv) simple fluidic control, (v) high sensitivity, (vi) small amount of measured sample, (vii) reagents stored within the card, (viii) manipulation of biomolecules (enzymes, DNA, or antibodies) as components of our LabCard, and (ix) thin fluidic devices able to be packaged within a Card. Basically, our strategy resides on making things as simple as possible through the combination of the bio-info-cogno-nano fields. This strategy is essential to make the LabCard word meaningful.

i) High throughput fabrication process

The device price depends on the volume production, therefore, the throughput must be very high, and consequently, wafer substrates might not be the final solution. **We believe that lamination and stacking by reel-to-reel processing of photopatternable dry films could be an excellent process to fabricate a LabCard.** We are exploring this approach that will allow us to manufacture LabCards through a reel-to-reel process.

ii) Few materials involved

Just one material should be used to monolithically integrate as many components as possible such as, micro-optical components, planar optical waveguides, microfluidic channels, microreactors, and sensor elements. The challenge is to find the right material. To put it briefly, the main features of this material are listed in the following table together with its consequent possibilities.

Seeking Features	Consequent Possibilities	SU-8
Low cost	Disposable devices	YES
Low surface energy	Ease fluidic movement	YES
Microfabricated structures	From sensing to fluidi& optical	YES
Planar technology	Easy to array and to immobilise	YES*
Dielectric	Electrical and thermal isolation	YES
Inert	Reliable over time	YES
Low auto-fluorescence,	Suitable for fluorescence	YES
Rigid channels	Tough microchannels	YES*
Tuneable refractive index	Easy to fabricate waveguides	YES*
Good adhesive properties	Sealing of the channels	YES
Biocompatibility	Suitable for bioreactions	YES*

Seeking features of the material, its consequent possibilities and suitability of SU-8 (* after modification by dilution or surface treatment)

Obviously, it is very difficult to find a material with properties that fulfil all these needs. Therefore, it would be very convenient to find a material that can be easily modified to adapt to those requirements [1,2,3,4]. Taking into account previous research works, they revealed that among the polymeric materials, SU-8, a thick-thin photoresist, spun on silicon, glass or plastic film, is a good candidate capable to fulfil almost the above mentioned materials requirements. This type of material has always been used as a high aspect ratio mask in etching processes and then stripped away. Therefore, there is an extensive list of recipes to obtain deep and narrow structures.

iii) Sample preparation

It is extremely difficult to obtain a LabCard that deals with really raw sample (food, blood, faeces or biopsy cells). Therefore, apart from incredible sample preparation devices, new sample collection approaches from end users and biochemists must be explored in extreme cases (i.e. meet juice instead of meet itself). Some remarkable miniaturisation achievements of a PCR have been achieved: thermocycling [5], cell separation [6], denaturation of DNA [[7]], PCR optical detection [8], and cell lysis [9]. Despite high miniaturisation, they have not integrated all functionalities to allow raw sample analysis as it is proposed here.

iv) Simple fluidic control

There is a lot of research being done in micro-fluidic machined devices[10]. Fluidic control devices, that need to be used more than once, will need to be externally actuated (i.e. magnetic fields, mechanical push pins). On the other hand, those that are used just once can be made from wax, hydrogels, and ferrofluidics or even frozen solvent. However, as far as we know, very few have been used in a commercial device.

It is crucial to mix the bio-info-cogno-nano pillars to make something simpler than it would be without this synergy. This compromise is being taken in order to make a true LabCard.

v) High sensitivity

Among all of the transducing mechanisms, the most sensitive is the optical one [11]. Therefore, this is the mechanism that will be used.

vi) Small amount of measured sample

Miniaturisation of biological assays has very well known advantages. However, it has a simple disadvantage, **the sample to be introduced in the LabCard is also miniaturised.** Consequently, the smaller the sample, the smaller the chance to detect the target, and in turn, the smaller the signal. For example, to detect Campylobacter in chicken by PCR, it is crucial to take at least one bacteria in the sample volume. Again, it is essential to coordinate the knowledge between biochemist and micro-nano engineers to work out a compromised solution.

vii) Reagents stored within the card

A true LabCard must contain the reagents stored in the reservoirs to facilitate its use. The challenge resides in the filling process, which should be parallel and arrayed to increase productivity. **The shelf-life of this card and its storage temperature depends on how reagents are stored.** New possibilities are being envisioned to increase that time such as, freezing reagents.

viii) Manipulation of biomolecules

Microengineers need to deal with and consider enzymes, DNA, nanobeads, or antibodies as components of the LabCard. It is necessary to be aware of the micro nano behaviour of these components (adsorption, biocompatibility, inhibition effects).

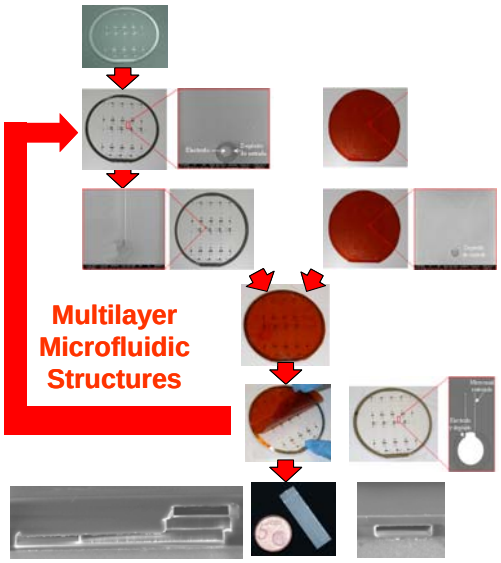
[1] Chun-Lung Wu, *et al.*, MicroTAS 03, pp. 1117-1120.
[2] S. Kragh, *et al.*, MicroTAS 03, pp. 1331-1334.
[3] B. Helbo, *et al.*, S&A A, 2004, vol. 111, no. 1, pp. 21-25
[4] N. Damean, *et al.*. Vol. 15, N 1, January 2005, Journal of Micromechanics and Microengineering.
[5] RH Liu; *et al.*, Anal. Chem. 2004, vol. 76, no. 7, pp. 1824-31.
[6] Perch-Nielsen, *et al.,* Lab on a Chip 3: 212–216.
[7] Oda et al 1990 /CRP/, Wittwer et al 1990 /CRP/
[8] M. Bahrami, *et al.,* In Proc. MME 04, pp. 203-206, Belgium.
[9] C.R. Poulsen, *et al.,J.* of Food and Protection, May 2004.
[10] Lammerink *et al.,* IEE-MEMS'96 , pp. 389-394, 1996.
[11] E. Verpoorte *et al.*, Proc. of the IEEE 03, Vol.91, No. 6.

(ix) Thin fluidic devices

A Card is an excellent candidate to be taken as a "capsule" to package a microfluidic device. In order to do so, a very thin microfluidic device must be fabricated.

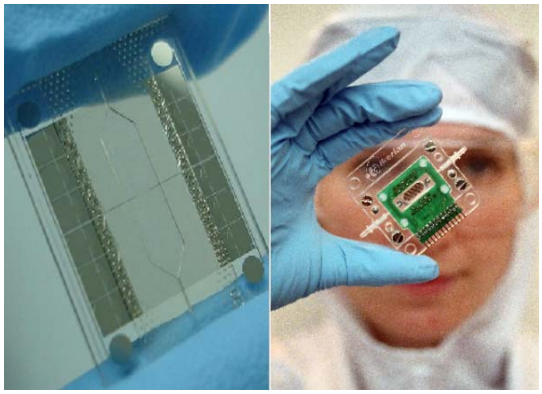
FIRST YEAR RESEARCH RESULTS

This section summarises our advances to fabricate a LabCard where sample preparation and detection take place. We have developed an approach that consists of using just one fabrication procedure to produce all Labcard components.



Fabrication process of SU-8 multilayer stack[12].

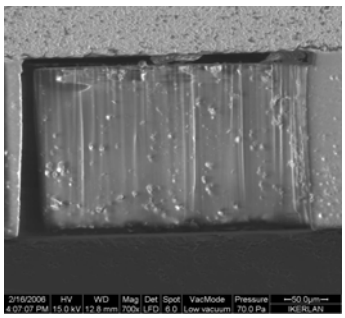
This procedure is based on the negative photoresist SU-8 to build fluidic control components, optical waveguides, electrodes, microfluidic channels and microreactors.



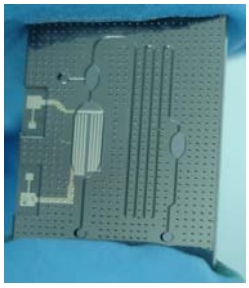
Picture of the SU-8 PCR chip.

Packaging capsule.

As for the packaging, the replacement of these devices within the capsule is simplified since there is no need of drilling to leave the inlets of the microchannels in contact with the outside world. A plastic capsule and O-ring per reservoir are enough to introduce liquid into channels.



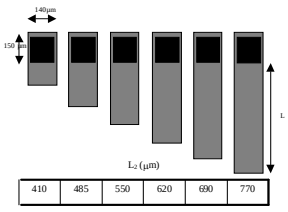
Embedded Cantilever



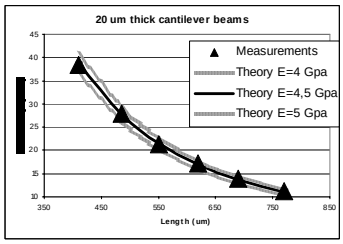
Electrolysis pump

Using this technology, we are getting preliminary fluidic control results. A cantilever check valve is being developed with an extremely low footprint (high density integration). An electrolysis pump is being characterised for fluidic pumping.

In order to obtain the Young´s modulus of the SU-8, measurements of the resonance frequency from different length of cantilever beams made of SU-8 were performed.

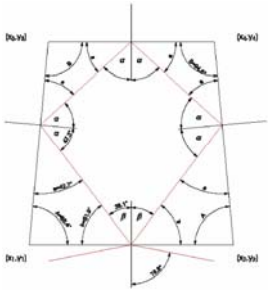


Design of six different cantilever beams

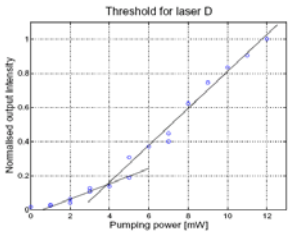


Resonance frequency measured and E modulus.

The singlemode laser has been reproduced and the spectrum has been characterized. We have found that lasers with gain lengths between 1000 µm and 4050 µm produces wavelengths in the interval 567 nm - 571 nm and that the output intensity was reduced compared with multimode lasers of the same size.



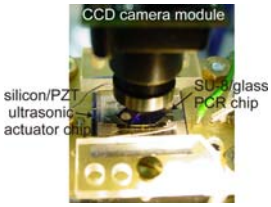
The trapezoid design of the lasers



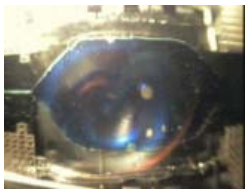
The normalised intensity of the dye laser

[12] M.Agirregabiria, F.J.Blanco, J.Berganzo, M.T.Arroyo, A.Fullaondo, K.Mayora and J.M.Ruano López. Fabrication of SU-8 multilayer microstructures based on successive CMOS compatible adhesive bonding and releasing steps . Lab on a chip, Vol. 5, No. 5, pp. 545-552

The mixer has been fabricated and tested. Complete mixing of two water-based fluids in the demonstrator of PCR chip has been obtained in 10 sec for 6 kHz vibration

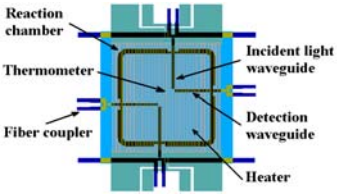


Picture of the Mixing stand-up

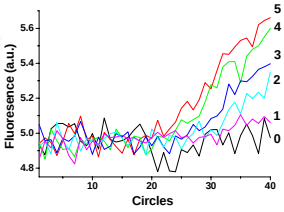


True picture of the mixed liquid in a chamber

Regarding the waveguides, this task has achieved remarkable results. A better result than expected since to our knowledge, this is the first time real-time PCR monitoring using integrated optical elements in a lab-on-a-chip system was demonstrated.[13]

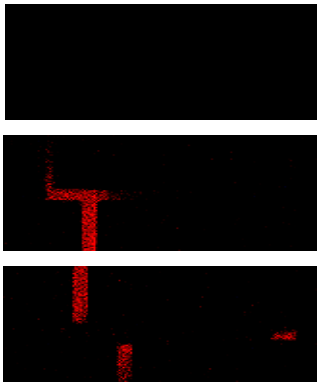


Schematics of the chip structure

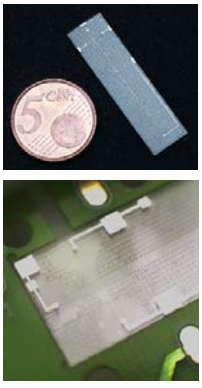


Real-time PCR on chip to amplify Campylobacter

The potential of the developed fabrication technology is also being demonstrated by the integration of a SU-8 Capillary Gel Electrophoresis of proteins.

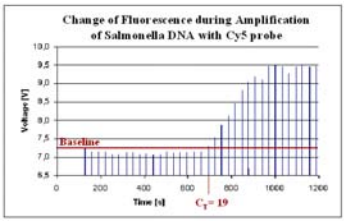


Results of a SU-8 Capillary Electrophoresis chip

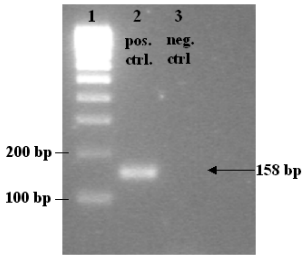


Another type of chip

This SU-8 technology transferred to a reel-to-reel process could be superior in cost, integration and ease of fabrication compared to glass, silicon and embossed bonding polymer devices. Our packaging technology allows easy handling and replacement of used LabCard.

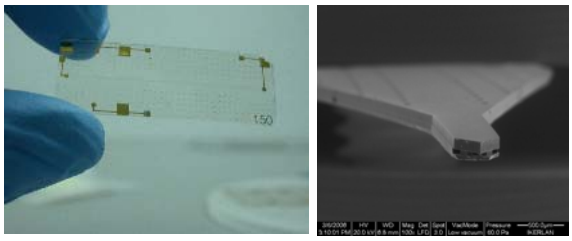


Real time PCR Salmonella *spp.* PCR on chip



Gel electrophoresis

As future work, we are developing a thin fabrication technology to manufacture complex microfluidic SU-8 only devices of 200 µm thickness. These devices could be easily packaged within a Card (see figure below).



New devices made of SU-8 only and 200 microns thick

To sum up, this fabrication technology allows research laboratories to produce devices within two days, offering an excellent tool for LabCard prototyping. Moreover, our 100% fabrication yield corroborates that this technique is feasible for mass production by lamination guarantying low cost and high reproducibility.

By using this process another Bio-Micro applications are being undertaken with fruitful results.[14]

CONCLUSION

The planned tasks are being developed according to the schedule. The successful fabrication process together with preliminary biological miniaturisation good results allow us to feel confident to tackle the rest of the project.

Apart from the technological achievements, the project has got public coverage by TV news, radios and newspapers. Besides, 8 conferences papers and 2 journals have been accepted. Two patents have been filled. Finally, a PhD student from Ikerlan will work at MIC during 2007.

[13] Z. Wang, A. Sekulovic, J. P. Kutter, D. D. Bang and A. Wolff. Real-time PCR detection Campylobacter jejuni on a microchip with integrated thermal system and polymer waveguides. Electrophoresis, Accepted.

[14] M. Agirregabiria, F. J. Blanco, J. Berganzo, A. Fullaondo, A. M. Zubiaga, K. Mayora and J. M. Ruano-López, Sodium dodecyl sulfate-capillary gel electrophoresis of proteins in microchannels made of SU-8 films, Electrophoresis,Vol 27, Issue 18, pages 3627-3634.