

The SmartBioPhone™, a point of care vision under development through two European projects: OPTOLABCARD and LABONFOIL

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This paper describes how sixteen partners from eight different countries across Europe are working together in two EU projects focused on the development of a point of care system. This system uses disposable Lab on a Chips (LOCs) that carry out the complete assay from sample preparation to result interpretation of raw samples. The LOC is either embedded in a flexible motherboard with the form of a smartcard (Labcard) or in a Skinpatch. The first project, OPTOLABCARD, extended and tested the use of a thick photoresist (SU-8) as a structural material to manufacture LOCs by lamination. This project produced several examples where SU-8 microfluidic circuitry revealed itself as a viable material for several applications, such as the integration on chip of a Polymerase Chain Reaction (PCR) that includes sample concentration, PCR amplification and optical detection of *Salmonella spp.* using clinical samples. The ongoing project, LABONFOIL, is using two results of OPTOLABCARD: the sample concentration method and the capability to fabricate flexible and ultra thin LOCs based on sheets instead of wafers. This rupture from the limited and expensive wafer surface heritage allows the development of a platform where LOCs are big enough to include all the sample preparation subcomponents at a low price. These LOCs will be used in four point of care applications: environment, food, cancer and drug monitoring. The user will obtain the results of the tests by connecting the Labcard/Skinpatch reader to a very popular interface (a smartphone), creating a new instrument namely "The SmartBioPhone™". All standard smartphone capabilities will be at the disposal of the point of care instrument by a simple click. In order to guarantee the future mass production of these LOCs, the project will develop a large dry film equipment where LOCs will be fabricated at a low cost.

Introduction

There is an urgent need to provide portable diagnostic tools to ensure rapid, affordable and simple analysis in many scenarios of our society (hospitals, airports, doctor's practice, roadside police controls, natural environment). The advance of Micro Electronic Mechanical System (MEMS) delivered some progress in this field. The 90's was a decade of incredible microfabrication process developments and transducing mechanism characterisation. However, few of these processes have been transferred

successfully into portable biological applications, mainly because of the lack of sample preparation integration and the difficulty to mass-produce the devices reliably. Currently, portable devices are based on low sensitivity immunochromatographic strips or low sensitivity electrochemical detection systems, whereas desktop systems are sensitive and automatic but bulky, heavy and expensive. Hence, owing to the new advances in dry film lamination and its synergy with Information Communication Technologies, it is now possible to prioritise and combine automatic sample preparation with low cost manufacture. This combination will create an intelligent and portable system across many sectors for efficient health treatment, high productivity food processing and secure environment monitoring.

This paper describes how this European research team was established and motivated. Furthermore, some basic information on the project together with the employed strategies, applications and technologies is given. Finally, a review of the obtained results is presented.

Consortium creation, projects' description and motivation

In order to achieve these goals, the first step was to establish a competitive and coherent consortium. This task, led by the Project Coordinator, was performed by collecting the contact information from a number of publications, conferences and

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webpages. After collecting all the information, phone calls and meetings were necessary to form the consortium and the suggested basic proposal. The next step was to find the relevant priority, topic and objectives in European Frame Work programs (<http://cordis.europa.eu/>). The final preparation step was the writing of a competitive proposal where the project is thoroughly described including its economic and social impact. Both OPTOLABCARD and LABONFOIL projects followed these steps achieving a clear European dimension and involving partners from both public and industrial sectors (research institutions and manufacturing companies). This combination provides a holistic view of the whole task of the value creation chain from the manufacture to the end-user of the developed LOC, including the economic-social impact, exploitation potential and viability of the results. This vision is crucial to succeed in the submission and negotiation phase with the European Commission, but more importantly, it produces a realistic research project.

The OPTOLABCARD¹ project was successfully submitted to a FP6 call. The project started in September 2005 and will be finished in February 2009 with the budget of 2.7M €. The partners involved were: IKERLAN-IK4 (coordinator), GAIKER-IK4 and Hospital de Donostia (*via* BIOEF) from Spain; DTU-Nanotech, DTU-Vet from Denmark; ITE from Poland; EVGroup from Austria; Microresist GmbH from Germany, and Silex from Sweden.

The second project, LABONFOIL,² is the sequel of OPTOLABCARD. The project was successfully submitted to an FP7 call, started in May 2008 with a duration of 4 years. The RTD centres involved in this proposal are IKERLAN-IK4 (coordinator), GAIKER-IK4, DTU-Nanotech, DTU-Vet, Politechnika Wroclawska-WEMiF, FhG for Photonic Microsystems, and Southampton University. Furthermore, the involved enterprises, *i.e.* Microresist GmbH, EVGroup, Biosensia, TATAA, Gema Medical and Biotools B&M labs have enough potential for further research and commercial exploitation of this technology. Finally, the Hospital de Cruces (*via* BIOEF), and NERC will carry out the prototype validation in real life situations in Health-Environmental-Food. The project budget is 7.1M €.

In order to provide cohesion and motivation to the consortiums, the researchers of OPTOLABCARD and LABONFOIL discussed the mission of these two projects reaching the following mission statement: "To create and demonstrate an innovative capability and economy in point of care systems for the benefit of society through rapid, ubiquitous and minimally invasive diagnostic devices for health and environment". On top of that, there is another social reason which is a driving force for both the consortiums: "The creation of the seed of a new industry". This could have an impact in the quality of life of citizens by creating a network of companies to manufacture and distribute The SmartBioPhone™.

Projects' strategy

The consortium partners have imposed a set of conditions in order to develop LOCs with future exploitation potential. Initially, the most important priority was to integrate the sample preparation within a chip independently of the cost. Then, the consortium's priority shifted to cost issues in order to obtain

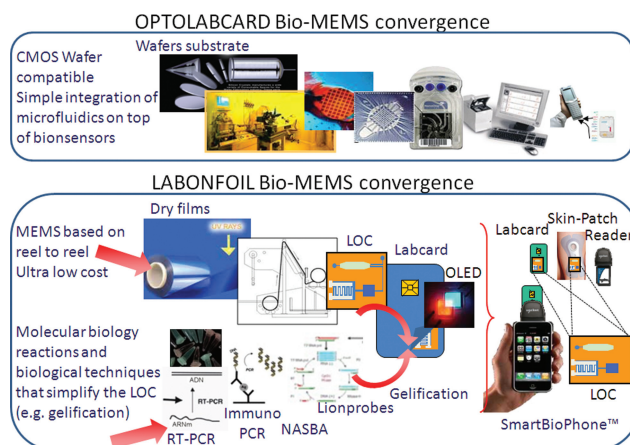


Fig. 1 Schematic description of the Bio-MEMS convergence and priorities of OPTOLABCARD and LABONFOIL.

a low cost fabrication method since typical microfluidic chips depended on expensive substrates (*e.g.* Pyrex or Silicon) (see Fig. 1). The wafer strategy is valid for microelectronics where chips are small in comparison with LOCs, and not disposable. Therefore, a compromise must be found between performance and cost. This is achieved by providing a set of convergence rules between the life sciences and the micro-nano technology. The applied convergence is described in the following sections.

Integration of sample preparation

In order to create a meaningful LOC, the integration of raw sample preparation is needed. Miniaturisation of biological assays has a number of known advantages. However, it also has a disadvantage: the sample quantity to be introduced into the LOC is also miniaturised together with the active area of the sensor. Consequently, the smaller the sample quantity, the lower the detection area/volume is, and in turn, harder to collect bio-signals and ensure high sensitivity. For example, to detect pathogens by PCR amplification methods, it is crucial to take at least one target gene in the sample volume. Consequently, the sample preparation should consist of a concentration step that flushes enough sample quantity through the chips and of a technique that places the target biomolecules on top of the biosensor surface.

Disposable LOCs

Point of care diagnostics must avoid any cross contamination between different samples. Therefore, it is crucial to select a manufacturing technique that produces cheap enough LOCs in order to make them disposable. The most popular low cost LOC fabrication method is injection moulding. For example, this technique has been successfully used to develop PCR prototype chips.³ Besides, there are some good commercial examples of the use of this technology in the form of a Lab on a Cartridge (*e.g.* Calliper). However, it still has not launched truly small and portable LOCs. The technology provides a low cost solution, but still has some disadvantages: (i) it does not provide embedded moveable components to create valves or pumps and, hence, include on-chip sample preparation, (ii) batch-to-batch

variation, (iii) low resolution (in comparison with photolithography), and (iv) difficulty to deposit other materials (e.g. metals).

In contrast to the inject strategy, the two projects presented in this article fabricate LOCs by lamination and photolithography. This combination provides unique advantages: all LOC advances can be transferred to a reel-to-reel technology and still exploit the complexity in shapes and resolution provided by photolithography. Hence, OPTOLABCARD provided an initial solution by laminating thick photoresist on standard wafers, ensuring CMOS compatibility, whereas LABONFOIL will produce thick photoresist LOCs without expensive substrate, decreasing dramatically the real estate cost of LOCs. These cost cuts are shown in the results section.

Integration of information society technologies

It is necessary to make LOCs easy to use. These two projects propose a way to achieve this goal by embedding the chip in a smartcard format, which can be inserted in a reader connected *via* USB (Universal Serial Bus) or SDIO (Secure Digital Input/Output) to a smartphone. Although SDIO is a less common interface, it is more convenient since it has a higher power limit than the USB standard.

This strategy will turn a smartphone into a portable diagnostic device offering a straightforward and vast range of communication, positioning and interface capabilities.

Simplicity as a rule

In order to establish LOC production lines, it is essential to simplify the LOC. The LOC must include the right biological reactions and be made of the least number of materials. Hence, complexity must be shared between biologists and engineers and not laid down on one side only. For example, in order to increase the detection signal of the system, an on-chip biological concentration stage or enzymatic amplification should be used. Another approach is the use of freeze drying and gelifying techniques to store the reagents within the chip, ready to use, facilitating the design, fabrication, handling, transport and storage of the LOCs.

Applications

The OPTOLABCARD project was focused on the development of a LOC that has two applications: detection of *Salmonella spp.* in human clinical samples and detection of *Campylobacter spp.* in broiler chicken production. On the other hand, the LABONFOIL project has a wider scope. The project aims at developing four LOCs with four different applications and one fabrication equipment (Fig. 2). The project will validate the developed LOCs in four fields:

- Labcard for marine algae analysis by NASBA to provide information about climate change, CO₂ sequestration, and toxic blooms.
- Labcard for detection of *Salmonella spp.* and *Campylobacter spp.* in broiler production (slaughter houses and farms).
- Labcard for monitoring biomarkers of colorectal cancer using a blood drop by immuno-Q-PCR detection.
- Skin patch to detect cocaine consumption by professional drivers.

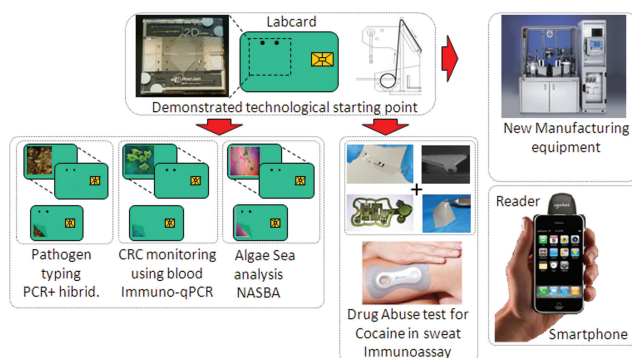


Fig. 2 Description of the equipment and applications. Three Labcards, a Skinpatch, a reader-smartphone and Dry film Lamination equipment.

Technology

These two projects are standing on the following technological pillars:

- Microfabrication based on dry film lamination and bonding technologies to mass produce polymeric microfluidic chips capable of being embedded in a Smartcard or skin patch.
- Ambient intelligence solutions based on smartcard and smartphone communications and human interfacing capabilities.
- Optical components for fluorescence detection based on hybrid components^{14,23} and/or organic light emitters and detectors.
- Molecular biology in microdevices like immuno-Q-PCR, real time PCR, reagent gelification, NASBA reaction, Lion probes and magnetophoresis.

Results

The OPTOLABCARD project delivered a methodology to fabricate and package a wide variety of LOCs made of a negative tone photoresist. This fabrication technology allows research laboratories to produce microfluidic channels on top of any biosensors fabricated on wafers within two days, offering an excellent tool for LOC prototyping production. Moreover, the 100% fabrication yield together with its lamination compatibility corroborates that this technique has a potential for future mass-production, guaranteeing low-cost and high reproducibility. The following sections will summarise some of the achieved results.

Methodology to fabricate LOCs

M. Agirregabiria *et al.* developed a method to integrate 3D microfluidic networks with biosensors to manufacture LOCs.⁴ Basically, the method consists of repeating the following steps: (i) photolithography of two negative photoresist layers to pattern the microchannels on a wafer, and the sealing cover on a polyimide film (*i.e.* Kapton), (ii) bonding of the two photoresist layers together, and (iii) a final releasing step of the polyimide film. The first photoresist process can be carried out on a silicon, pre-processed silicon, polymeric or glass wafer. After these three steps, more SU-8 layers on polyimide films can be prepared, bonded and released, yielding 3D multilayered SU-8 stacks. The estimated manufacturing cost of SU-8 microfluidic circuitry on top of a biosensor with integrated platinum electrodes would be approximately 2 € cm⁻².⁵

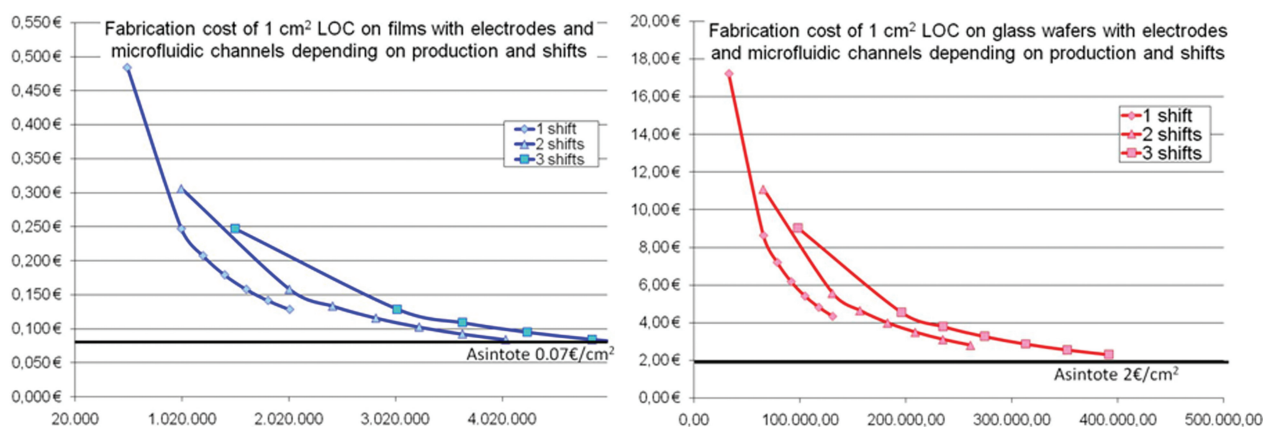


Fig. 3 Manufacturing cost of a LOC of 1 cm² versus production and working shifts. The cost is dramatically reduced if the bottom rigid wafer is substituted by another dry SU-8 layer on a flexible substrate.

This manufacture process can be modified by substituting the rigid bottom wafer with another SU-8 coated polyimide film. This new process will create an SU-8-only device, since both top and bottom substrates are released at the end of the process from the polyimide films (See Fig. 3). Detailed information about this process can be found elsewhere.⁶ Using this technique, the manufacturing cost decreases dramatically to 0.07 € cm⁻² whereas the wafer approach is 2 € cm⁻².⁵

Methodology to detect fluorescence bio-signals

Due to sophisticated construction and incompatibility with LOCs, none of the typical fluorescence detection methodology and equipment can be directly applied in OPTOLACARD project to co-work with developed LOC. Therefore, a simple, cheap and sensitive optical fluorescence detection system was developed to measure real time PCR^{23,7}. It is based on the application of a semiconductor low-cost laser and an array of CCDs to excitate and collect the fluorescence. The hardware part of the read-out system co-works with a specialized software for conditioning, processing and visualization of analysed data to give analysed information to the user.

Integrated examples

This fabrication concept created several fabrication versions to be applied in different applications. Many of them made use of this fabrication to monolithically integrate SU-8 microfluidic circuitry on top of biosensors or, in other cases, to create different SU-8 microfluidic circuits on top of wafers. For example, an SU-8 microchannel was integrated on top of a Mach Zehnder interferometer fabricated in silicon.⁸ A SDS-CGE chip was successfully tested to separate proteins on a glass wafer⁹ and on polymethylmethacrylate (PMMA) wafer.¹⁰ A flow sensor has been designed and fabricated.¹¹ SU-8 free-standing structures embedded in microchannels for valves and pumps have been designed¹² and fabricated.^{13,14} A SU-8 microfluidic circuitry was monolithically integrated on top of a magnetoresistive immunosensor¹⁵ to detect *Escherichia coli* O157:H7. A SU-8 LOC performed the purification and concentration of 1 mL of raw sample plus to a final volume of 2.6 µL for real time PCR.^{16,17} The concentration method was characterised obtaining a 90%

bead capturing efficiency at a realistic flowrate (1 mL min⁻¹).^{18,19} PCR reagents were also freeze-dried within this PCR chip.²⁰ Other devices were developed using the mentioned alternative method, where the rigid substrate is substituted by another flexible film,⁶ This technique was used to fabricate SU-8 microfluidic chips⁵ and microneedles for drug delivery in *ex vivo* neural tissue²¹ and for ischemia monitoring in a rat kidney.²²

Packaging of LOCs

Since the LOCs should be disposable, it is essential to use a package which allows an easy replacement of the used LOCs.

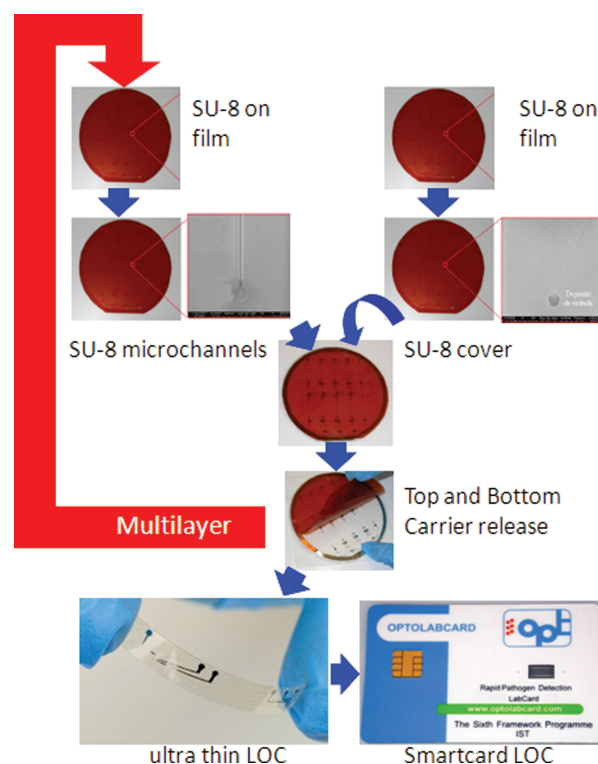


Fig. 4 Scheme of the fabrication process for the manufacture of SU-8 only devices. Both the micro channels and the cover layers are fabricated using a polyimide film as a substrate.

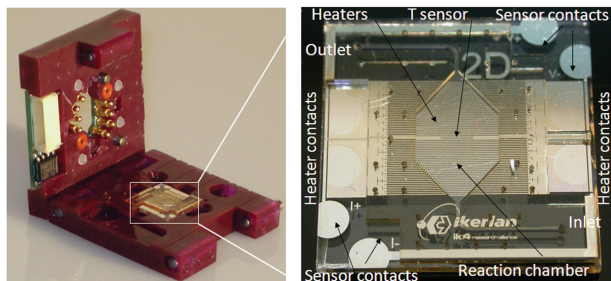


Fig. 5 Pictures of the packaging and the PCR chip where sample concentration and PCR takes place.^{16,17} This package allows an easy replacement of the LOCs since there is no glue or wire bonding.

This package or capsule must include the proper fluidic, electrical and optical connections to the macro world without the need for adhesives or wirebonding. The multilayer approach LOC fabrication simplifies the packaging. All layers are photopatternable, therefore, the inlet and the outlet of the microchannels are kept in contact with the outside world (Fig. 4). This fact avoids slow drilling or etching steps to the cover, radically simplifying the packaging. Hence, a plastic capsule with external flexible tubes and a low cost O-ring per reservoir are enough to insert liquids into channels, as can be seen in Fig. 5.

Spin off company

As a result of sound achievements of this project, a company namely microLIQUID²² was created. This new company provides a set of standard LOCs together with customised fluidic solutions, based on the fabrication principles obtained through the OPTOLABCARD work.

Conclusions

This paper describes two European projects aiming at providing packaged LOCs that include sample preparation plus detection at a low cost. The aimed instrument named SmartBioPhone™ consists of a handheld base unit (smartphone and reader) and a disposable Labcard or Skin patch with a LOC inside. In order to obtain a commercial instrument, some constraints are self-imposed: total integration of sample preparation, LOC disposability, use of advantageous Information Society Technologies and balanced complexity of both bio-applications and micro-engineering sides. Two manufacturing solutions are presented. The first one provides a technique to integrate 3D microfluidic circuitry on top of biosensors, and the second one allows the fabrication of big and cheap LOC's to carry out complex sample preparation steps. Furthermore, the successful range of different developed LOCs and components under development indicate the potential of this LOC fabrication platform.

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