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# Silicon Chip Technology – Electrophoretic DNA Analisys

M. SIMION[1], I. KLEPS[1], F. CRACIUNOIU[1],
L. SAVU[2], M. MIU[1], A. BRAGARU[1]

[1]National Institute for Reserch and Development in Microtechnologies (IMT), Bucharest, 077190, Romania
E-mail: monicas@imt.ro

[2]DEXTER com.SRL, 021021, Bucharest, Romania
E-mail: lorand_savu@yahoo.com

**Abstract.** The development of miniaturised devices for genetic diagnosis is of great interest for medicine applications. In this paper, we focus on the design, the experimental technology and characterization of a new silicon micro-bio-chip for rapid testing of DNA material. This chip integrates two classical processes for DNA analysis: polymerase chain reaction – PCR – technique for fragments amplification and electrophoresis for separation of DNA fragments respectively. The proposed device was realized on silicon substrate and it consists of two reservoirs connected by one channel and two electrodes inside of reservoirs; the backside electrical circuit allows the control of temperature variation. The structures were tested using human DNA and the corresponding analyses time was less than 45 min.

**Keywords**: biodevices, silicon microfabrication, DNA analysis.

## 1. Introduction

The microfluidic devices are emerging around the world as new tools to characterize biological events and samples and are generic named Laboratory-on-a-Chip systems. They offer new solutions for fast, specific, automated and high throughput analysis. The components have potential for assembly into complex, low-power, integrates analysis system at low unit cost.

A polymerase chain reaction (PCR) is a classical technique used to amplify a specific gene or region of DNA. Since its development, PCR has become one of the most useful and versatile methodologies applied to molecular biological applications. PCR applications range from diagnosis of infectious diseases to analysis of specific gene mutations that occur in a variety of genetic disorders. The current PCR protocol state-of-the-art requires several hours for simple analyses, as well as large sample volumes (25 to 50 μL). The protocol is prone to several technical error levels, and results can be muddied by exogenous DNA. Also, regarding to DNA fragments separation, there are several methods used in biological laboratories; the most frequent are based on electrophoretic separation in agarose gel or capillary electrophoresis. These methods have some disadvantages: need a certain quantity of biological material, strong voltage and long time to assure a good separation.

Recently, a number of groups have explored miniaturization of PCR amplification and integration of online detection using the micro-electro-mechanical system (MEMS) technology. Therefore, the availability of portable, reliable instruments that are capable to facilitate the DNA analysis will have an enormous impact on a broad spectrum of applications such as rapid medical diagnostics and point-of-use agricultural testing [1].

## 2. Device design

Our studies are concentrated on development of a new device on silicon for genetic analysis. The design and fabrication processes of the microchip with patterned microreservoirs take advantage of materials and processing tools of silicon microfabrication [2]. In the same time, using standard processes from silicon technology we propose the integration of specific electrical circuits on the same structure, which allows both the DNA amplification and electrophoretic separation necessary to achieve the complete analysis.

The proposed microchip consists of two reservoirs connected by one channel and the specific electrical circuits; in Fig. 1, there are presented the schematic views of the structure with integrated system for PCR and electrophoretic analysis:

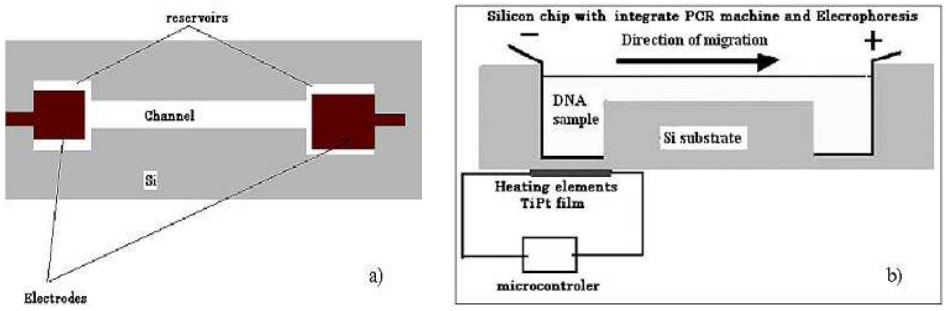


**Fig. 1.** Schematic representation of structure: (a) top view of the integrate system; (b) side view of the PCR reaction chambers and the microfluidic channel, where electrophoretic separation of DNA fragments will take place.

Two types of structures were designed: first with a straight channel and second one with a serpentine channel in order to increase the length of channel. The width of the channel was 25 μm in both cases.

The first reservoir has on the back side a temperature control unit consisting of heating element and thermal sensor, and inside one electrode; the second reservoir contains the second electrode. Using a COVENTORWARE simulation programme the heating element geometry – Ti/Pt resistor – was chose to assure the proper thermal cycle for DNA amplification [3]. The electrode pair integrated into microfluidic system will resolve the DNA fragments separation stage. In order to realize the closed structures for fluidic applications, and also to facilitate the optical detection, the Si patterned substrate was bonded to a glass wafer.

## 3. Experimental realisation

From technological point of view, the system fabrication is based on standard processes used in silicon device manufacturing, such as: silicon etching, metallic and dielectric film deposition. The substrate material was a double side polished p-type (100) silicon wafer, with 6–10 Ω·cm electrical resistivity and 450 μm thickness. Both Si wafer sides were subjected to technological processes: (i) one side contains the microfluidic system – two microreservoirs connected through microchannels and the electrical circuit for DNA electrophoresis; (ii) the second side contains the heating element. Starting from these designed elements, the device fabrication technology includes a set of 3 photolithographic masks: (i) for the first side micropatterning: M1f – microfluidics system etching windows; M2f – electric circuit for DNA electrophoresis; (ii) for the second side micropatterning: M1b – electric circuit for temperature control unit on the second Si wafer side.

The following processes were performed for test structure fabrication; (1) thermal oxidation at 1 100°C in in oxygen and water vapor ambient; (2) photolithographic process using M1f to define channels and reactors; (3) isotropic etching process of silicon using dioxide mask; (4) photolithographic process on the back side of the wafer to define the resistors structures (M1b); (5) $SiO_2$ etching using a photoresist mask; (6) deposition of Ti/Pt films (50/200 nm) by vacuum cathodic pulverization; (7) Ti/Pt lift-off process in order to obtained heating elements; (8) photolitographic process for electrodes definition using mask M2f; (9) Cr/ Au deposition (50/150 nm) by vacuum cathodic pulverization; (10) Cr/Au lift-off process.

The Si micromachining was realized by wet etching process through a 1.8 μm silicon dioxide obtained by thermally oxidation of Si wafer in oxygen and water vapor ambient. The Si etching process is the main step of the technological flux. A photolithographic positive process was used to define the hard mask for selective Si etching. Etching solution can be alkaline (anisotropic etch) or acid (isotropic etch). Some aspects, which were taken into consideration, are pointed out: (i) the anisotropic etching process realized in KOH solution at 80°C lead to the deterioration of the silicon dioxide mask with a lower rate as silicon etching rate, but the superficial geometry is modified for long etching times; (ii) during the isotropic etching, the HF solution

penetrates through the silicon dioxide mask, and the silicon walls present a stepped profile. In the same time, the etching profile of silicon walls obtained in alkaline solutions is anisotropic and rugged, compared to isotropic and smooth in acid solutions, but in this case the channels are enlarged due to the underetching phenomena [4].

Our previous experience [5] allows us to choose the proper parameters – type, concentration and temperature of etching solution. In order to obtain higher depth/width channel ratio an isotropic etching process in a solution prepared as a mixture of acids, 8 parts of nitric acid, 3 parts of acetic acid and 3 parts of hydrofluoric acid was selected. Silicon dioxide mask is low resistant for this mixture, but is enough to etch a channel with a deep of about 150 microns. The etching reaction being very exothermic one, for better control of the etching rate and to preserve as much as possible the $SiO_2$ mask, the temperature during etching process was monitored. During the etching process, the etching solution was cooled using a cooling bath containing ice with water mixture. In this way we obtained the necessary depth for the channels.

In Fig. 2 there presented optical images of the both proposed microfluidic systems with details for the corresponding microchannels:

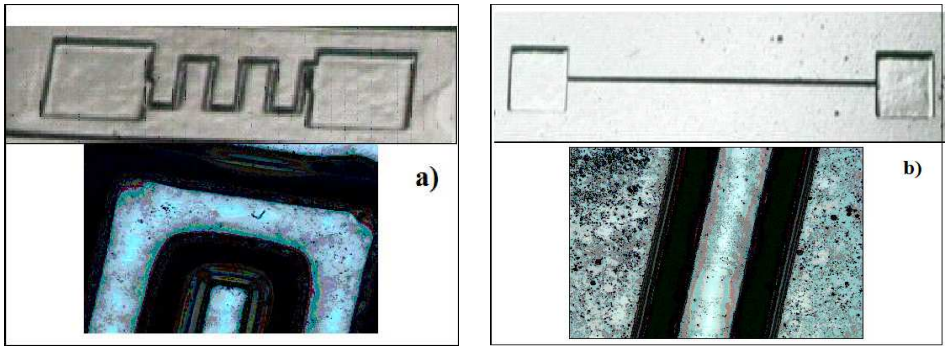


**Fig. 2.** Optical images of test structure after etching process;
a) structure with serpentine channel and detail.

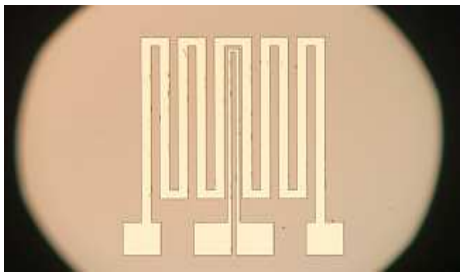

**Fig. 3.** Heating element and thermal sensor with contact pads.

The electric circuit for temperature control unit was realized on the back side Si wafer, using the following technological processes: the oxide was removed and both sides were spin coated with photoresist and the masks pattern was transferred. The

resistor material was deposited on the whole back side of the Si surface. We have used a metallic sandwich Ti (500 Å) / Pt (2000 Å) prepared by vacuum cathodic pulverization which assure the adhesion on Si substrate [3]. In Fig. 3 is presented an optical image of the heating elements obtained after the lift-off process. The gold (Au) electrodes and pads were deposited by vacuum cathodic pulverization process on the bottom side of the reservoirs.

## 4. Results

First biological experiments using this test structure were realized using external Pt electrodes for DNA electrophoresis. The thermal cycling in a PCR device involves three temperature slopes: 90°C to 95°C for DNA denaturation, 50°C to 65°C for DNA hybridization, and 70°C to 77°C for DNA replication [6, 7]. The cycle is then repeated until a sufficient amount of the DNA sample is obtained (usually 20 to 40 cycles) [8].

The amplified PCR product, labelled on-column with an intercalating fluorescent dye, is injected in the capillary for electrophoretic analysis. The two sets of primers were used to amplify an unique section of either the X or Y chromosome. The PCR mixture consists of: 1×PCR buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP and 2.5 units of Taq polymerase, 0.25 µM each primer and 5 nG of template DNA. After amplification, the sample was injected into the CE separation channel by applying field strength of 125 $V \cdot cm^{-1}$ and separated by applying field strength of 250 $V \cdot cm^{-1}$. The migration process was analysed using a fluorescent microscope with suitable filters. Figure 4 show the fluorescent signal obtained along the channels on optical fluorescent microscope. Successful sex determination using this PCR reaction from human genomic DNA is demonstrated in less than 45 min.

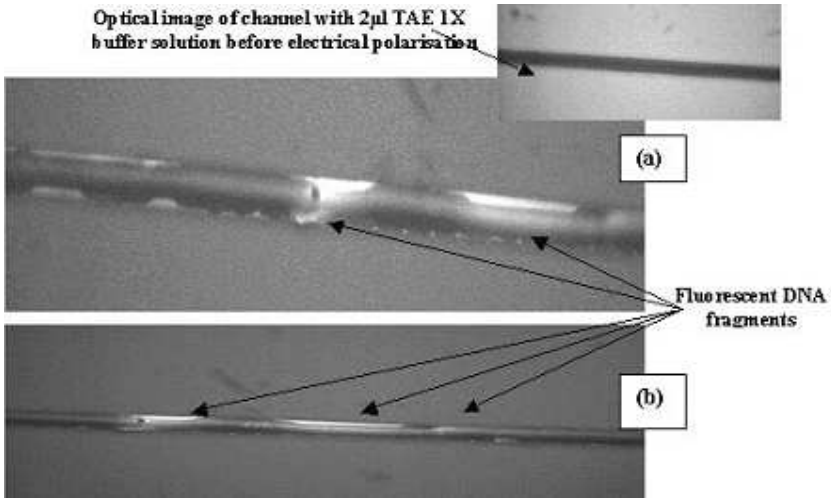


**Fig. 4.** Optical fluorescent image of DNA fragments separated along two channel type by electrophoresis: (a) 250 µm, (b) 50 µm.

The proposed design allows the monitories of amplification process evolution for the target sequences that take place in the first reservoir connected to a microfluidics system, in specific thermal conditions.

## 5. Conclusions

The standardized interconnections between each component in our device, coupled with the inexpensive photolithographic techniques, should be ideal for the low-cost production of integrated devices. However, important technical challenges remain, including maintaining minimal liquid evaporation and solute adsorption, increasing detector sensitivity, and controlling complex fluidic and electronic operations.

The presented silicon device had integrated microfluidics using embedded channels, DNA bearing electrodes, heat resistors and temperature control. The results presented in this paper demonstrate the technological and biological compatibilities of the device and give preliminary results of PCR and electrophoresis analyses. Microarray probes and microfluidics technologies were used to realized the proposed devices.

Finally, the low voltages and power used in this device suggest that simple handheld battery operation is both feasible and practical. The functionality of the device was demonstrated by rapid testing human DNA.

Also, the PCR chip can be integrated in a more complex system like lab-on chip; by decreasing the ADN analysis time, the quantity of reactants is reduced and the costs decrease.

The portable device is intended as an instrument for fast PCR with detection capabilities integrate. The availability of simple devices that analyze DNA without the need for specialized laboratories, elaborate equipment, or highly skilled personnel should yield benefits across many fields, including medical diagnostics, forensics, and agricultural testing.

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