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Competitive technology approaches for Electronic Hybridisation Detection in a microsystem with microfluidics for diagnosis genetic tests

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***Abstract*—This paper is presenting competitive technology alternatives for the electronic hybridization detection in a microsystem with microfluidics for diagnosis genetic tests that are carried out by two competitive research projects. The technologies developed are a photosensor, a capacitive sensor and an optical real-time affinity biosensor. The performance of those biosensors will be evaluated but also their manufacturability and cost will define the appropriateness of each one for industrialization and their integration on a microsystem for diagnosis genetic testing.**

I. Introduction

A critical parameter in order to achieve maximum discrimination between fully matched hybrid and a single-base mismatched hybrid is the accurate control of the thermal conditions at a microarray interface. This has been accomplished so far through the integration of external heaters and thermal sensors. For SNP detection, a method based on the temperature gradient on a polymer microfluidic chip has been reported [1]. However, in all cases there is the risk of the thermal denaturation of surface-immobilized DNA or DNA present in free solution. Therefore, novel platforms for on-chip hybridisation assays should be established with incorporated heaters and thermal sensors, which will allow control and rapidly change of the temperature during on chip hybridization

The alternative technologies that will be developed in the research presented here will be innovative and they will be fully integrated in a miniaturised, low-cost bio-microsystem for genetic tests with an embedded micro fluidics system. The microfluidics part of the microsystem under development will be implemented using both alternative silicon and polymer material. Both developments will include in their on-chip micro fluidic part sub-systems for, extraction, purification and amplification steps, including temperature and flow monitoring sensors for the control of those sub-systems. The electronic detection part of the microsystem for all the alternatives is on silicon material with integrated sensors and their readout for detection and control.

The first alternative is optical, and is interesting for its high sensitivity. It is an innovative implementation where the optical detector will be integrated in the chip. The second detection method is mechanical and based on the capacitive detection of the deformation of a thin silicon membrane when molecules are hybridized on its surface. Its advantage is that it does not require a labeling step of DNA. However, the challenge here is to improve the sensitivity which is *a priori* lower than the optical method. Finally the third electronic detection is based on an innovative electronic DNA hybridisation detection sub-system using a patented optical real-time affinity biosensor. The first two detection technologies are under development in a research activity (Micro^{2}DNA) that is developing an innovative, fully integrated, bio-microsystem for genetic analysis including also the microfluidics part. on polymer material. The last electronic detection technology will be investigated in another research activity (BioMicro), that is also integrated with embedded micro fluidics based on silicon substrate. Speciallised instrumentation will host the Microsystems under development and is presented also in this paper.

II. Optical real-time affinity biosensor

The main obstacle in realizing silicon optocouplers is the integration of efficient and fast light emitters on a Silicon wafer through a CMOS compatible process. Recent advances in light emitting Silicon nanoscale devices have raised new hopes. However, stability issues remain to be solved. On the other hand, in realizing a monolithic optical sensor the efficiency of the light source is not as important an issue compared to stability and reproducibility concerns. The light emitting devices to be employed here are silicon avalanche diodes which are known to emit light when reverse biased beyond the breakdown voltage. Such devices are fabricated by standard silicon processing steps. The detectors are p/n junctions optically coupled to the light emitters by Si_3N_4.waveguides, as shown in Fig.1. The critical element here, is the way the Si_3N_4 fiber is aligned to the emitter and the detector and the way it bends from the field oxide to the end points of the optical link: Since both the light emitter and the detector are planar devices, efficient coupling to them is achieved by bending the fiber so that it contacts both devices under a normal angle (Fig. 1). Because a small radius curvature on a waveguide can cause substantial losses of light, SiO_2 spacers are created at the emitting and receiving ends to minimize the losses. This is achieved by a thick (2 μm) TEOS deposition over the thermal field oxide, lithographic patterning and reactive ion etching in CHF_3 to induce vertical walls. Subsequently, another thick TEOS deposition follows and similar etching (with no patterning) creates the spacers.

The spacers are shown in Figure 1. An overall field oxide thickness of 2.5 μm assures relatively smooth bending and enough distance between the long horizontal segment of the fiber and Silicon interface to minimize substrate losses.

The most critical point for efficient optical coupling is the precise alignment of the light emitter to the fiber. Here, the lightemitting junction is self-aligned to the up-going vertical segment of the fiber by ion implanting the emitter with boron through the Si_3N_4 fiber film. The vertical segment of the fiber along with the spacer masks the P^{++} implantation in a way that

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the avalanche junction lies exactly under this segment The N^+ side of the avalanche junction is already implanted before the spacer deposition. The light emitted towards and within the critical angle of the up-going vertical segment is trapped within the fiber and waveguided all the way to the detector. There, the abrupt breaking of the fiber at the vertical segment/silicon interface assures effective detection even for small diode lengths (as opposed to leaky mode detection).

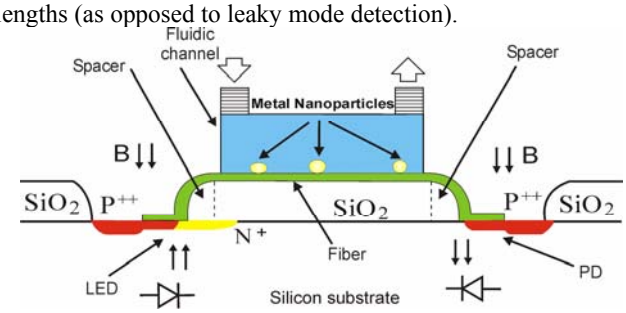


Figure 1: Monolithic optocoupler. Schematic of the optocoupler emphasizing the emitter, the detector, the waveguide and the bending spacer

The energy of the P^{++} Boron implantation is a function of the Silicon Nitride thickness. This thickness is chosen in the range 150-200 nm to optimize sensitivity. The implantation energy is selected so that a good part of the implanted Boron ions will penetrate the horizontal segment of the fiber. At the same time, the selected energy assures that the ions will stop at the upright segment of the fiber at the edge of the spacer to form the self-aligned junction for both, the light emitter and the detector. The dose of the pre-existing N^+ Phosphorus implant on the detector side determines the breakdown voltage of the avalanche diode and is selected so that the breakdown voltage is below 10 V. The device outlined in the previous lines is fabricated through standard Silicon processing steps including oxidation, implantation, annealing, film deposition by LPCVD, plasma etching, metalization, and passivating oxide deposition.

Signal transduction is made possible by the labeling of the analyte biomolecules that bind to the probe immobilized on the fiber. These analyte molecules are labeled with chromophore groups or metal nanoparticles and their interaction with the waveguided modes results in attenuated total reflection and intensity loss at the detector. Therefore, the detector photocurrent drop is a measure of the analyte conentration in the sample. Despite the low quantum efficiency of the silicon LEDs, detector photocurrents of 1 nA are obtained for LED excitation currents of 10 mA. Given the low detector leakage current (< 1pA) and capacitance (<1 pF), such photocurrents can be detected with a high degree of accuracy. That was demonstrated in the IST-BIOMIC project where sensitivities in biomolecular detection of 20 fM were demonstrated [2].

Since temperature control and stabilization is important to obtain DNA hybridisation specificity, a Pt heater will be lithographically defined in the form of a metal line near and in parallel with the fibers. The thickness of the Pt resistor will be chosen so that the resistance is in reasonable values considering the number of optocouplers required and the total length of the heater. The heater at the same time serves as a temperature sensor by monitoring its terminal voltage at a constant current and observing how the resistance changes with conditions. The control electronics will adjust the drive Pt heater current so that the temperature remains in the desired range. A thin insulating film will passivate the heater so that no electrolysis is induced on the sample. The LEDs and detectors will be protected by the cladding layer and other overlayers including polymer films that will define the fluidic area where the sample will react with the DNA probes. These probes will have already been spotted on the exposed fiber length. This way, real time measurements of the hybridisation is possible without cross talk effects that might have interfered in other types of transducers.

III. PHOTOSENSOR ELECTRONIC DETECTION

Several protocols for microarray based SNP and mutation analysis have been developed (reviewed in Syvanen et al 2001) and the selected approach in the current project consists in tiled arrays (Cutler, Zwick et al. 2001) since these allow both photo- and capacitive- detection for comparison purposes. Tiled arrays involve the generation of an array of oligos that vary in specific positions in order to create perfect matches to the fragmented DNA molecules which will bind strongly or mismatches that will result in weaker binding.

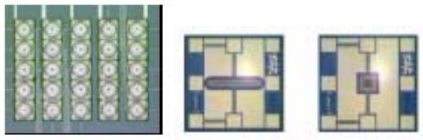
In the photo-detection context, tiled oligonucleotide arrays are suitable for single colour detection (Cutler, Zwick et al. 2001). The fragmented DNA molecules are labelled with a fluorophore probe and the more or less binding pairs result in relative intensities of the oligo spots that have to be compared. This requires the same amount of functional oligo to be deposited (by spotting) or synthesized (Hughes, Mao et al. 2001) at each spot. The aim is to minimise variations in the amount of arrayed oligo, which will impede the analysis of single colour intensities.

Optical Detection is one of the primary methods used for detection of hybridisation events, and in particular when fluorescence is used. The optical setup includes an excitation light source, typically LEDs or a laser, optical filters to separate the excitation light wavelength for the fluorescence wavelength and a detector. There is a range of microarray scanners available for scanning and detection of DNA microarray based platforms. The lowest cost and least sensitive is a CCD (or CMOS) based imaging system, where the whole microarray is illuminated with the excitation light source and image processing is used to determine the results. Alternatively a laser scanning based microarray scanner can be used. In this configuration a laser beam is raster scanned across the micro array device. The fluorescence is collected via appropriate optics and filters into a PMT (Photomultiplier tube). A PMT is an extremely sensitive light detector and may be operating in photon counting mode, where individual photon of the fluorescence light are counted. The extremely sensitivity of the PMT leads to better results compared to the CCD sensors, but the PMT has the drawbacks of being expensive and slower than the former. In biochip based DNA detection solutions optical detection is generally performed using optical detectors such as Photodiodes (PDs) or PIN Diodes. These devices give an analogue signal which is proportional to the optical signal detected. However their sensitivity is limited. There are no miniature photons counting sensors available with a form factor appropriate to biochip architectures. PMTs are large bulk glass tube based devices requiring high power (1000s Volts) and are not suitable for such applications (Table 1).

Technology	Operating Voltage (V)	CMOS Compatible	Sensor Diameter (mm)	Dark count (cps)	Timing jitter (ns)	Array size	Quantum efficiency (%@670nm)	Robustness
PMT* (vacuum tube)	>1000	No	5 – 10	<500	<1ns	8 x 8	20-30	Fragile vacuum tube /damaged by ambient light
1st gen. silicon+	200	No	0.18	25-500	<500ns	1 x 1	65	damaged by excessive light
2nd gen silicon#	30	Yes	0.01 - 0.1	1-500	<150ns	64 x 64	45-80	not damaged by excess light

Table 1 :Review of photon counting technologies

In this context, a miniature photon counting optical detector solution is a significant enabling tool for biochip based DNA detectors, enhancing the sensitivity of the optical detector by many orders of magnitude. This means that the quantities of DNA could be reduced significantly, the light emitting parameters of the fluorescence labels can be reduced resulting in the availability of new labels, and opening the possibility of direct detection of particular molecules. SensL are developing a 2D array (See Figure 2,a) of photon counting sensors on a single chip that will enable detection of images of fluorescent hybridised DNA samples. It utilises the high speed operation and low light level detection capability of the 2nd generation silicon detectors, the Geiger Mode-APD. These devices produced using CMOS compatible processing are low power as appropriate for POC and portable applications and will have a low cost base. A detailed description of these Geiger Mode-APD detectors may be found in [3].



(a) (b)

Figure 2: *Array of photon detectors developed by SensL. (b) Different design shapes*

SensL has already show that optimal performance can be achieved (reduced dark count) by optimising the shape of the sensor to the application.(Figure 2,b). Thus, the array of sensors can be designed in such a mode to improve the signal/noise ratio of the measurements with a form factor adapted to the spots into the integrated device. Moreover, the optical interface between the detectors surface and the buffer solution plays a critical role in the collection of the photons emitted by fluorescence (Figure 3). The propagation of the photons to the detector can be improved by adding an appropriate configuration of optical layers. These may be either thin optical coatings of dielectric materials or porous silica gel layers. In both cases, the DNA probes are grafted on the surface of the sensor by a covalent link and the chemical fictionalisation needs to be taken into account in the design of the optical layers. Thus doing, the total flow of photons emitted in the solid angle which is "seen" by the detector increases. In the case of discrete photo detectors (see Figure 2), this is a crucial advantage.

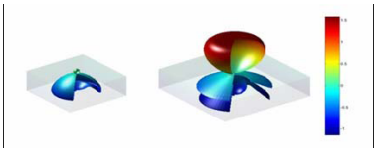


Figure 3: *Emission of fluorescence photons at the interface between a glass slide and a buffer solution*

Then the new developments in silicon photon counting detectors can eliminate costly optics and high power sources from medical equipment and provide higher levels of sensitivity at the same time. The use of photon counting detectors and procedures will generate a wealth of new point-of- care and point-of-use instrumentation customised for the individual within the required detection environment.

IV. CAPACITIVE SENSOR ELECTRONIC DETECTION

MEMS sensors are based on mechanical movements and deformations of their micromachined components, such as single-clamped suspended beams (cantilevers), double-clamped suspended beams (bridges), or suspended diaphragms.

In capacitive detection, displacement is measured as a change in the capacitance of a plane capacitor. The technique has been applied in the fabrication of a capacitive type AFM probe where the cantilever is one of the capacitor plates [5]. This technique is highly sensitive and can provide absolute displacement, but becomes difficult when applied in electrolyte solutions because of the faradaic currents between the capacitor plates thus limiting its use in biosensing applications. Moreover the technique permits for the integration of the electronic interface circuit together with the sensor. In order to take advantage of the high sensitivity of capacitive detection, the fabrication of functionalized ultra thin silicon membranes is proposed. The membrane will physically separate the solution from the inside cavity isolating the capacitor plates. A microarray of such sensors will be built allowing for multiple concurrent DNA hybridization.

An alternative approach for the detector array based on the stress induced on a thin silicon membrane due to reactions between the receptor DNA deposited on the membrane surface and the sample under investigation will be explored in the third group of tasks in the second workpackage. This kind of detectors have been successfully been applied in biological applications employing silicon cantilevers and optical or piezoresistive detection. Capacitive detection could challenge the sensitivity and flexibility achieved by both of these techniques.

Up to now capacitive sensing in biosensing applications has been of limited use as it is not well suited for measurement of large displacements and does not work in electrolyte solutions because of the faradaic currents between the capacitor plates. Therefore, despite the high sensitivity of the technique, it is of limited use in biosensing applications and it is for this reason that optical and piezoresistive detection have been favoured.

Capacitive DNA Sensors Arrays based on the exploitation of surface stress changes and subsequent bending of an ultra silicon thin membrane are to be fabricated. The membrane will seal the capacitor plates from the electrolyte solution thus enabling capacitive detection.

A capacitive biosensor microarray will be developed. In this array each element of the array will be a capacitor comprised of an ultra thin silicon membrane suspended over a cavity and a counter electrode on the substrate. Operation of the device will rely on the induced stress due to the reaction between the receptor DNA a number of ultra thin silicon membranes covering a shallow cavity formed into a silicon dioxide layer etched on a silicon substrate containing the counter electrode of the capacitor detector. Silicon fusion bonding will be used to transfer the membrane over the cavity. A patented by the proposers process will be used for membrane fabrication. In Figure 4: ***Hybridazation Process*** the basic idea is illustrated. The

hybridization process (b) results in membrane deflection due to the change of the surface free energy that eliminates the need for attaching labels to detect specific binding. Special provision will be taken so that the device accommodates for the microfluidics to be incorporated on the system.

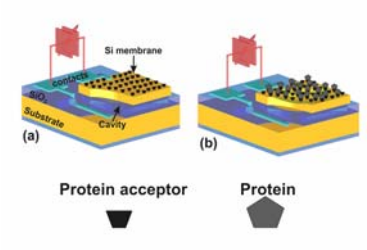


Figure 4: Hybridazation Process

V. Instrumentation Unit for the Biosensors

As new advances in detection techniques are achieving unprecedented levels of maturity, more innovative NAT instrumentation can be developed to fulfil the market needs for laboratory instrumentation and medical diagnostics applications. The introduction of new devices is highly driven by the readiness level of competing detection technologies (i.e. photosensors, capacitive sensors, or optical real-time affinity sensors), their performance (i.e. reliability, sensitivity, specificity), and how they may influence the final cost per test. Based on this, instrumentation designers are obliged to find an optimum balance between these factors to adequately respond to the end-used needs in cost-effective ways. The instrumentation of choice shall provide an effective biosensor platform allowing its control, readout of detection signals, and test interpretation that will eventually lead to a correct diagnostic. The miniaturization of fluidic management techniques along with low-cost detection sensors open an era of disposable biochips that will automate NAT assays from sample to result in a nearly operator-free mode. Figure 5 illustrates a representative instrument envisioned for the point-of-care diagnostic test environment. The main characteristics of these instruments are the small footprint, the user-friendly interfaces, the automation of the testing protocols, and most importantly the capability of a single instrument to run multiple assays by only using a different biochip. Hence, when doctors will introduce a biochip for a particular assay into instrument docking port, the instrument will self-acknowledge the type of test to execute and will automatically generate a result. Furthermore, the high risk of cross contamination always present in NAT, is greatly reduced by the use of biochips since all steps from sample preparation to detection are performed within the biochip itself, which also minimizes instrument contamination.

Figure 5 : Representative instrument for NAT envisioned for point-of-care applications

Depending on the application these instruments may have different characteristics. Table 2 shows these possible differences.

	Low-throughput Instruments (Micro^{2}DNA)	**Medium-throughput Instruments** (BioMicro)
End-User Environment	Doctor/practitioner office	Hospital laboratories, critical care units, emergency rooms
Type of test	"yes" or "no" tests	Complex tests
Data Manager	If any, external to instrument	On-board decision-making tools
Connectivity to LIS	Limited connectivity	Enhanced connectivity
Throughput Level	Individual assay biochip (few test per day)	Parallel assay biochip (up to a hundred tests per day)
Instrument Size	Handheld, small footprint	Desktop, medium footprint

Table 2: Biochip-based instrumentation options

VI. Conclusion

Three competitive technologies for electronic hybridisation detection of an integrated microsystem for diagnosis genetic tests were presented. The first two detection technologies are to be integrated on a polymer based biochip including DNA extraction and amplification that is based on a modular microfluidic architecture. The third electronic detection technology will be integrated in a future development of a fully silicon microsystem (with microfluidics as well on silicon) for genetic analysis. The instrumentation unit for the control and readout of the Microsystems was presented also in this paper.

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