## PROJECT III.2 BIO-MICROSYSTEMS

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**Objectives**

- Development of bioanalytical lab-on-a-chip devices based on monolithic optoelectronic transducers (bioactivated optocouplers).
- Development of monolithically integrated interferometric biochips for label-free biosensing
- Development of white light interferometric setup for label free monitoring of biomolecular reactions.
- Develop highly sensitive and/or label free assays suitable for point of care applications

**Funding**

- EU, FP7-ICT, STREP, "PYTHIA", Monolithically integrated interferometric biochips for label-free early detection of human diseases (start 01-05-2008, duration 42months), www.pythia-project.eu

## MAIN RESULTS IN 2011

**A: Monolithically integrated interferometric biochips for label-free biosensing**

The progress of integrated optical structures, such as waveguides and gratings, has allowed the implementation of various evanescent wave sensors which found wide application in real-time monitoring of biomolecular interactions offering high sensitivity, and fast response time. Among the evanescent field sensors, the Mach–Zehnder Interferometric (MZI) biosensor is one of the most promising devices due to its high sensitivity and accuracy. In the framework of the PYTHIA project a novel approach, **Broad-Band Mach-Zehnder Interferometry (BB-MZI)** (fig. 1) is explored as an alternative operation principle **based on a monolithically integrated biosensor array that is fabricated by standard silicon technology.** This radical concept is suitable for the **early diagnosis of human diseases through the label-free, multi-analyte detection** of gene mutations and proteins.

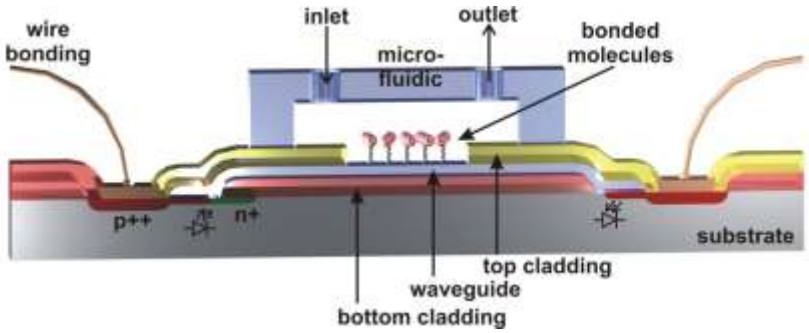


***Fig. 1.*** *Cross section of the basic sensor concept with the integrated light source, planar waveguide (sensing arm) and photodetector.*

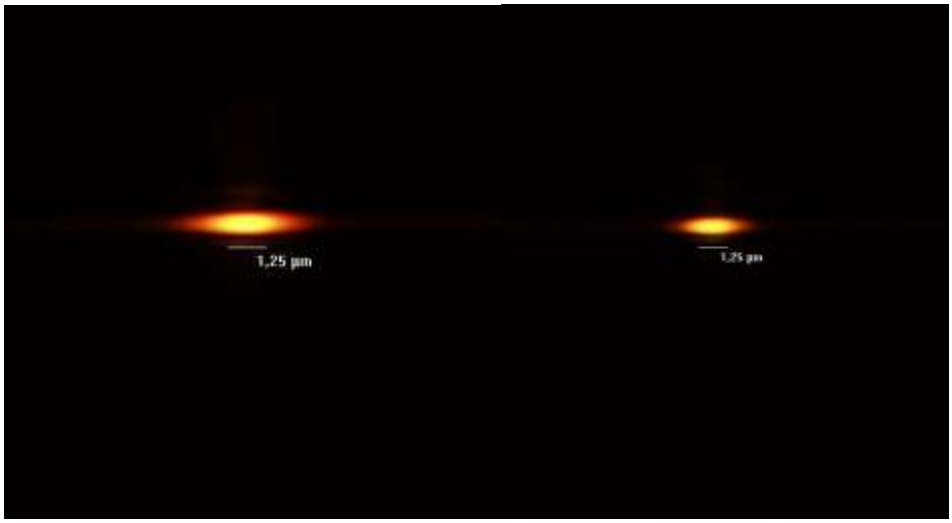


***Fig. 2**. CCD image of the waveguided fundamental mode: (left image) TE and (right image) TM.*

The PYTHIA optoelectronic transducer is based on the monolithic optocoupler platform that has been developed and patented[1] at the Institute of Microelectronics. The optocoupler consists of waveguide self-aligned to a VIS-NIR light source (avalance photodiode) and a photodetector. The monomodal waveguide, fig. 2, is patterned to a Mach-Zehnder interferometer and its principle of operation is the spectroscopic interference due to the optical path difference originating by biochemical events. This way all necessary active and passive optical components are integrated on the same chip resulting in a miniaturized optoelectronic lab-on-a-chip. The integrated nature of the basic biosensor scheme allows for the development of arrays tailored to specific diagnostic applications. Each biosensor array is comprised of individually functionalized light source/optical waveguide series coupled to a single detector for multiplexing operation. Currently, each chip carries 10 sensors in an area of less than 40mm$^2$, fig. 3, and is the smallest multi-sensing truly integrated optical transducer ever made This layout allows for two chip configurations: the fully integrated where the output signal is photodetector's current and the semi-integrated where the photodetector part is diced-off and the detection is performed through an external spectrometer.

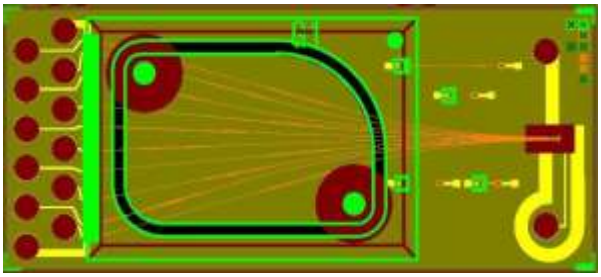

***Fig. 3.** CAD design of assembled chip. LED contact pads are on the left-hand side while detector is at the right hand side. The ten waveguides (orange colour) are coupled to the LEDs at the one side and at the other side merge to the common photodetector. The common fluidic cell (light green colour) for all sensors with the inlet and outlet ports is placed on top over the sensing areas.*

Encapsulation with an appropriately designed microfluidic system performed at wafer-level scale (Jobst Technologies GmbH) allow for the easy delivery of the samples to be analyzed and ensure the facile contact with the external low-noise electronic components. The encapsulated

[1] *a) K. Misiakos, S. Kakabakos "Integrated optoelectronic silicon biosensor for the detection of biomolecules labeled with chromophore groups or nanoparticles", PCT WO2007/074348, US7319046 b) K. Misiakos, S. Kakabakos, I. Raptis, E. Makarona, "Integrated optoelectronic silicon biosensor for the detection of biomolecules labeled with chromophore groups or nanoparticles", OBI, 1006509 2008 c) I. Raptis, K. Misiakos, S. Kakabakos, P. Petrou, E. Makarona, M. Kitsara, "Monolithically Integrated Physical Chemical and Biological Sensor Arrays based on Broad-band Mach-Zehnder Interferometry", PCT WO2009/115847 A1 (2009)*

array is fixed on a cartridge (fig. 4), ready to be manually inserted to its final position in the housing, where it is directly connected to the optical and electrical interconnects. Measurements are performed through the light-weighted measuring apparatus developed by VTT, fig. 5. Thus unit provides all the necessary power, control and read-out electronics and external fluidic connections and components in order to supply the necessary reagents and perform the measurements through especially developed software.

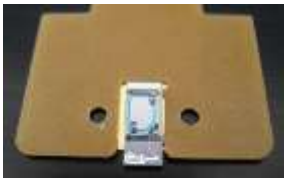

***Fig. 4.*** *Picture of a chip holder frame made with attached brass foil and chip*

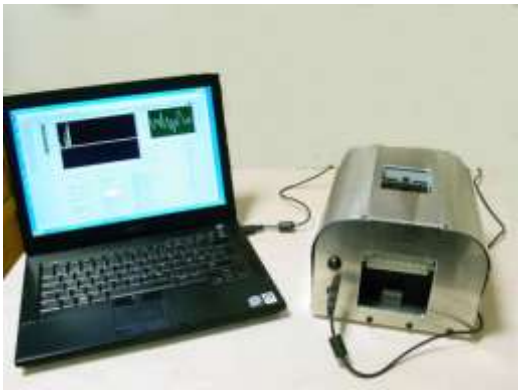

***Fig. 5.*** *PYTHIA measuring apparatus*

The performance of the PYTHIA device was evaluated with models assays but also in particular applications concering concerning the:
a) Quantitative determination of free- and total-PSA for early diagnosis of prostate cancer.
b) Detection of selected RET mutations diagnosing predisposition for MEN2
c) Detection of known PRPF31 and RPE65 mutations aiming at efficient diagnostic tools for Retinitis Pigmentosa.

The evaluation was performed using appropriately spotted chips after packaging and dicing using the prototype PYTHIA measuring apparatus installed at IRRP NCSR-D, fig. 6.

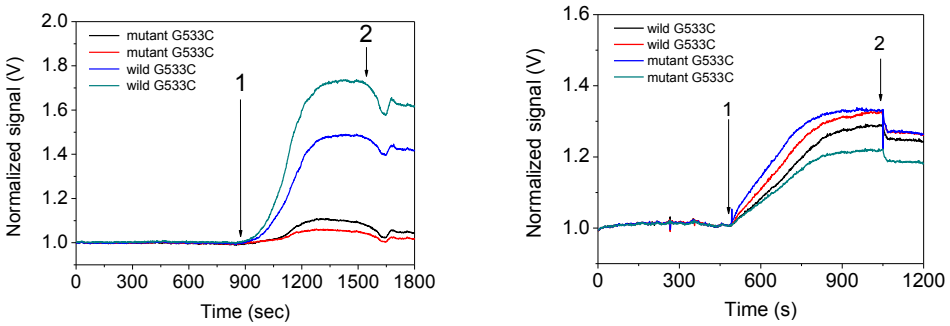


***Fig. 6.*** *Response from chip spotted with wild- and mutant-type sequences corresponding to G533C RET gene mutation during hybridization with: Left: PCR product obtained from the DNA of a wild-type individual; and Right: PCR product from a heterozygote carrying the mutation. Fluid sequence was as follows: start to 1: 5xSSC (hybridization buffer); 1 to 2: PCR product; 2 to end: 5xSSC.*

**B. Microfluidics and Microarrays (see detailed description in Project I.2)**

Significant work has been done in both microfluidics and microarrays and is described in detail in Project I.2. In the area of microfluidics, the following 3 topics were pursued:

I. Flame Aerosol Deposition of TiO2 Nanoparticle Films on Polymeric Microfluidic Devices for On-Chip Phosphopeptide Enrichment (see Fig. 7a,b). This work is a continuation of our previous work on wet deposition of $TiO_2$ in polymeric microchips.

II. Controlled protein adsorption on rough microchannel walls (see Fig. 7c). Here we demonstrate significant selectivity between large protein adsorption on rough plasma treated hydrophilic microchannels, compared to rough plasma treated superhydropohobic channels.
III. Continuous-flow microfluidic device for DNA amplification (μPCR) on flexible polyimide substrate (see Fig. 7 d). We have designed and implemented an on-chip μPCR unit.

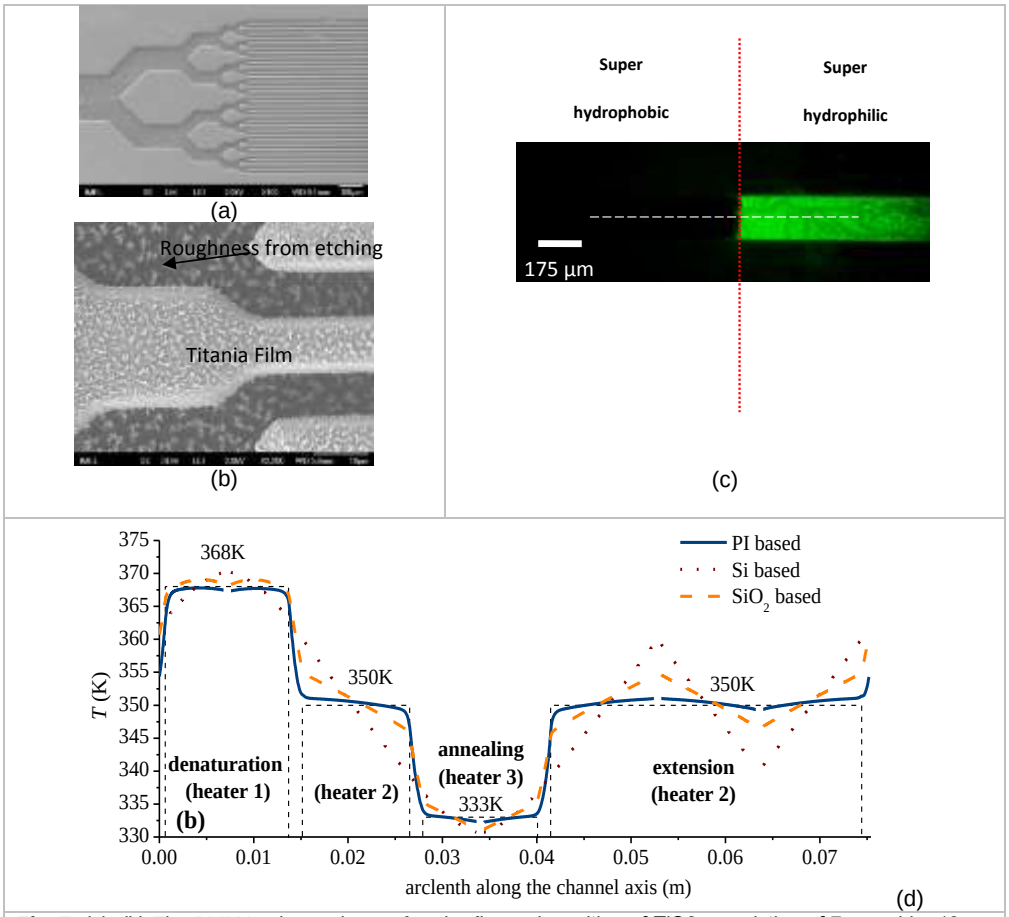


***Fig. 7.*** *(a), (b) The PMMA micro-column after the flame-deposition of TiO2, consisting of 7 μm wide, 12 μm deep microchannels. Notice that the microchannel bottom surface is rough and the walls in between are flat. The particles (white) cover both the walls and the microchannel bottom. The deposition is uniform over large areas. (c) Fluorescence image of a microchannel with patterned wettabilty (from superhydrophobic, contact angle CA~150º, to superhydrophilic, CA<10º) formed in PMMA by $O_2$ plasma etching, deposition of a hydrophobic Teflon-like film in the left zone, and adsorption of AF488 labelled goat anti-rabbit IgG antibody. (d) Temperature along the channel axis (simulation) for three different device materials (PI, $SiO_2$, Si). The inlet velocity is 1 mm/s for a μPCR chip*

In the area of microarrays, two new technologies were developed and demonstrated for high capacity, high density, high intensity microarrays

I. Highly dense and uniform protein and DNA microarrays through photolithography and plasma modification of glass substrates (see Fig. 8 a)
II. High Capacity and High Intensity DNA Microarray Spots Using Oxygen-Plasma Nanotextured Polystyrene Slides (see Fig. 8 b)

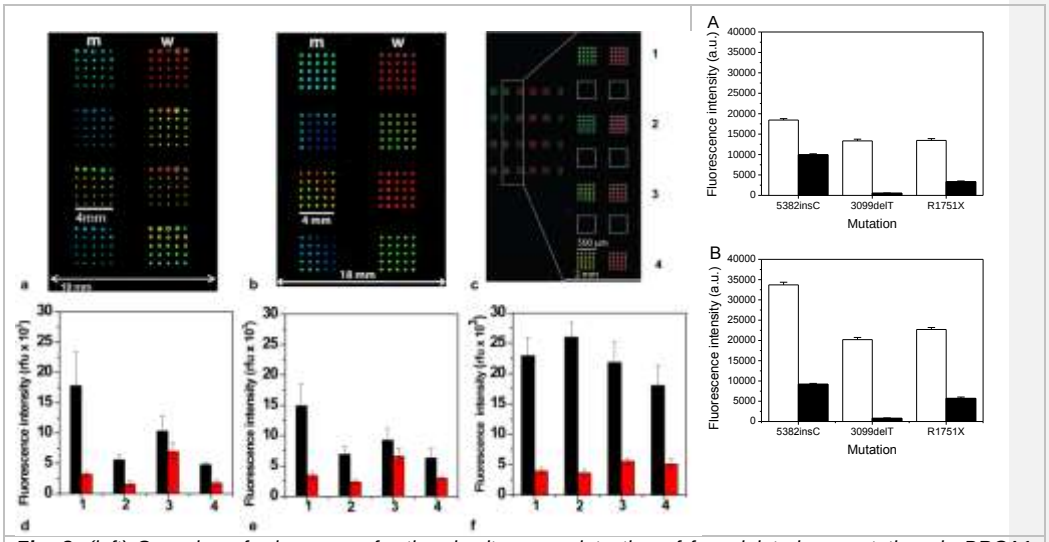


***Fig. 8.*** *(left) Scanning of microarrays for the simultaneous detection of four deleterious mutations in BRCA1 gene. The wild- (w) and the mutant-type (m) corresponding to 1: G1738R, 2: 5382insC, 3: 3741insA and 4: 3099delT mutation were spotted on commercial (a) PS slide (b) epoxy modified glass slide, and our developed (c) Ormocomp/glass slide. Fluorescence intensity values for the spots corresponding to wild-type (black columns) or mutant-type (red columns) of the four BRCA1 mutations obtained from (d) PS slide (e) epoxy-glass slide, and our (f) Ormocomp/ glass slide shown to outperform the commercial ones.*
*(right) Fluorescence signals from microarrays created by spotting wild- (white columns) and mutant-type sequences (black columns) corresponding to 5382insC, 3099delT and R175IX BRCA1 gene mutations on an untreated A (top) and 10-min $O_2$ plasma treated PS slide B (bottom)) after hybridization with fluorescently labelled oligonucleotides complementary to the wild-type sequences. The arrays have been washed sequentially with HEN buffer dilutions from 1 to 0.063 HEN at 30$^0$C and distilled water at 4 $^o$C. Each point is the mean value of 30 spots ± SD. Notice the better discrimination on the plasma treated slides.*

Detailed description of the above can be found in Project I.2 Micro and Nanofabrication using lithography and plasma etching.

## PROJECT OUTPUT IN 2011

### Publication in International Journals

1. "Real-time monitoring of nanomolar binding to a cyclodextrin monolayer immobilized on a Si/SiO2/novolac surface using white light reflectance spectroscopy: The case of triclosan" Author(s): D. Maffeo, Z. Velkov, K. Misiakos, K. Mergia, A. Paulidou, M. Zavali, I.M. Mavridis, K. Yannakopoulou, JOURNAL OF COLLOID AND INTERFACE SCIENCE, 358-2, Pages: 369-375, 2011

### Conference Papers

1. "Monolithically Integrated Frequency-Resolved Mach-Zehnder Interferometers for Highly-sensitive Multiplexed Label-free Bio/Chemical Sensing" K. Misiakos, A. Botsialas, I. Raptis, E. Makarona, P. Petrou and S. Kakabakos, G. Jobst, R. Stoffer, M. Hoekman, IEEE Sensors 2011 (Limerick, Ireland, 10/2011)

### Invited Presentations

1. "Monolithic Silicon Optocouplers for Bio-Chemical Sensing", Konstantinos Misiakos, Eurosensors 2011 (Athens, Sept. 4-7, 2011)
2. "Monolithically Integrated Mach-Zehnder Biosensors for Real-time Label-free Monitoring of Biomolecular Reactions", E. Makarona, P. S. Petrou, A. Bourkoula, A. Botsialas, M. Kitsara, S. E. Kakabakos, R. Stoffer, G. Jobst, G. Nounesis, I. Raptis, K. Misiakos 33rd Annual Int. Conf. IEEE EMBS (Boston, USA, 09/2011)