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 white light interferometric setup for label free monitoring of biomolecular reactions.
- Develop highly sensitive and/or label free assays suitable for Point of Care and Point of Need applications

FUNDING

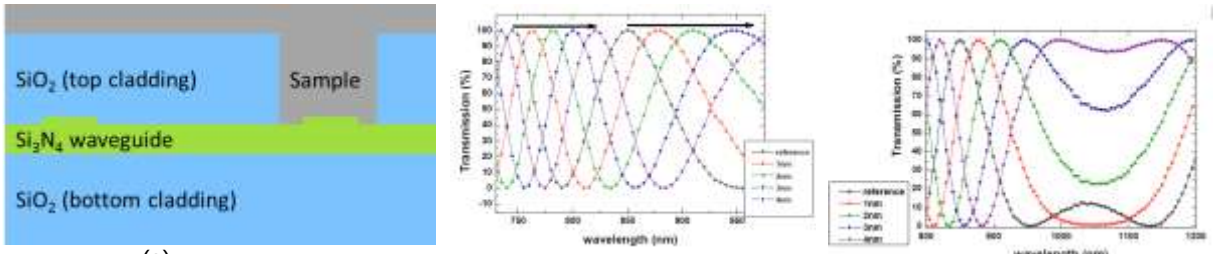
- EU, FP7-ICT, STREP, "FOODSNIFFER", Monolithically integrated interferometric biochips for label-free early detection of human diseases (start 01-09-2012, duration 36months), www.foodsniffer.eu

MAIN RESULTS in 2012

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), fig. 1a biosensor is one of the most promising devices due to its high sensitivity and accuracy. In the framework of the European funded FOODSNIFFER the **Broad-Band Mach-Zehnder Interferometry (BB-MZI) approach** that has been patented[1] by our group is combined with an on-chip spectrum analyzer and miniaturized and self-aligned LEDs all **monolithically integrated on the same Si chip and manufactured by mainstream silicon technology.** This radical concept is going to be applied at the Point of Need detection of harmful substances in food and in particular of certain pesticides, allergens and mycotoxins.

[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)*

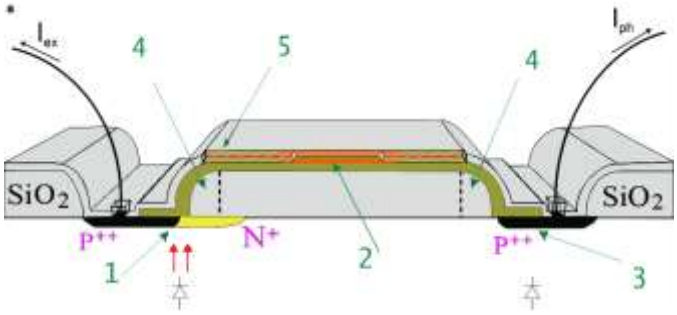


(a) (b)

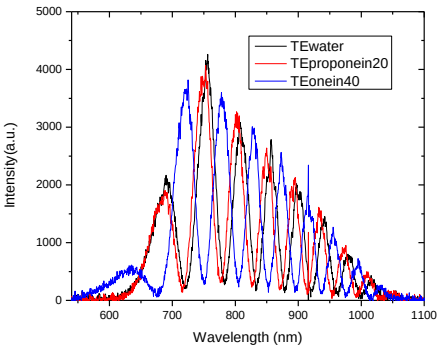
Fig. 1. *a) MZI structure. The cladding layer over the sensing arm is removed and is appropriately functionalized. The core of the waveguide is appropriately structured to be monomodal. b) Simulation of transmission spectra for BB-MZI device upon binding of target molecules with an equivalent thickness in the 0-4nm range. The thickness of the silicon nitride layer is 150nm.*

The FOODSNIFFER optoelectronic transducer is based on the monolithic optocoupler platform that has been developed at the Institute of Microelectronics. The optocoupler consists of a waveguide self-aligned to a VIS-NIR light source (avalanche photodiode) and a common photodetector, fig. 2a. By dicing off the photodetector part, fig. 2b the transmission spectrum could be monitored by an external spectrometer coupled to an optical fiber aligned in front of the chip facet, fig. 2c.

In the course of the FOODSNIFFER project, the monomodal waveguide 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. The output of the BB-MZI photonic structure is coupled to a miniaturized spectrum analyzer and an array of photodetectors. 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 particular chip will be the only one integrating such optical and electronic devices offering that way a unique miniaturized optoelectronic platform for sensing applications without any compromise in sensitivity. The integrated nature of the basic biosensor scheme allows for the development of arrays tailored to specific diagnostic applications. Each biosensor array will be comprised of individually functionalized light source/optical waveguide series coupled to a single spectrum analyzer and a photodetector array.



(a)



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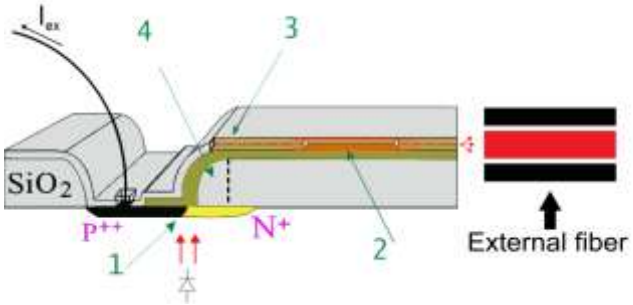

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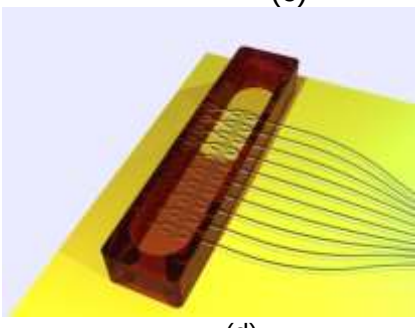


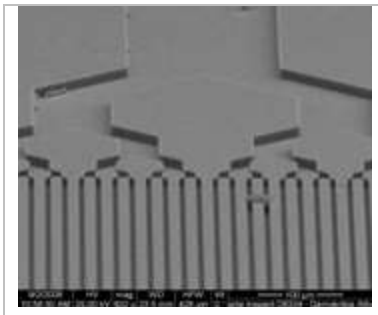
(d)

Fig. 2. *a) Schematic of the monolithic optocoupler. Light emitting diodes (1) and detectors (3) are optically coupled through a self-aligned silicon nitride layer (2) while the spacers (4) provide for the smooth waveguide bending. The nitride layer is shaped as Mach-Zehnder interferometer (5) and the top cladding layer over the sensing arm is removed and functionlized for the targeted analytes. b) Semi-integrated version of the chip showing the cleaved emitting edge of the integrated waveguide optically coupled to an external fiber connected to a USB powered portable spectrometer. c) Spectral shifts as a result of a cover medium change. The waveguides are photonically engineered so that an increase in the effective refractive index of the sensing waveguide will cause a blue shift. The black curve correspond to water, the red one to a propanol/water mixture of 1/20 (R.I. change 3.6X10^{-3}) and the blue one to a propanol/water mixture of 1/40 (R.I. change 1.8X10^{-3}). Sensitivities in excess of 15 μm/RIU are obtained and Limits of Detection (LOD) of 1.510^{-6} RIU. Here, the exposed arm length is 2 mm. d) Schematic detail of the FOODSNIFFER chip showing the BB-MZI array and the microfluidic channel on top for the supply of the sample to be analyzed.*

B. Microfluidics and LOC (see detailed description in Project I.2, section D2)

Significant work has been carried out for both microfluidics and microarrays for bioanalytical applications and is described in detail in Project I.2, section D2.
In the area of microfluidics, the following 3 topics were pursued:

I. Phosphopeptide Enrichment and Separation in an Affinity Microcolumn on a Silicon Microchip: Comparison of Sputtered and Wet-Deposited TiO_2 Stationary-Phase (see Fig. 3a,b,c). This work is a continuation of our previous work in polymeric microchips.
II. Continuous-flow microfluidic device for DNA amplification (μPCR) on flexible polyimide substrate and integration in a Lab-On-Chip platform (see Fig. 3d,e). Improvements over previous design were implemented and device efficiency for PCR was demonstrated.
III. Comparison of pressure drop and flow field in Superhydrophobic and superhydrophilic, hierarchical, plasma-nanotextured polymeric microchannels.



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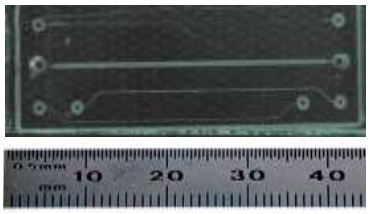

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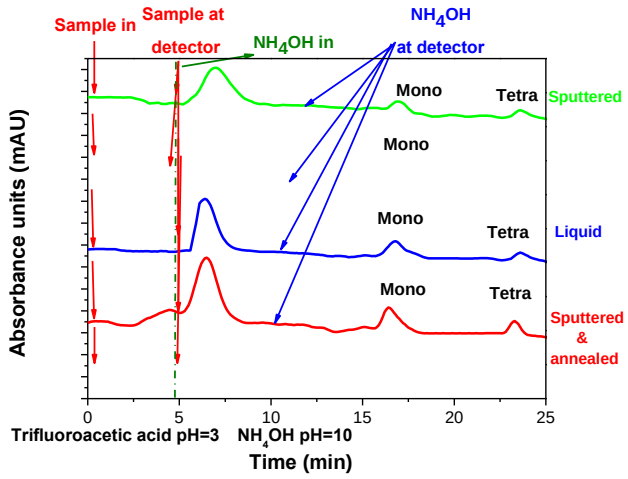

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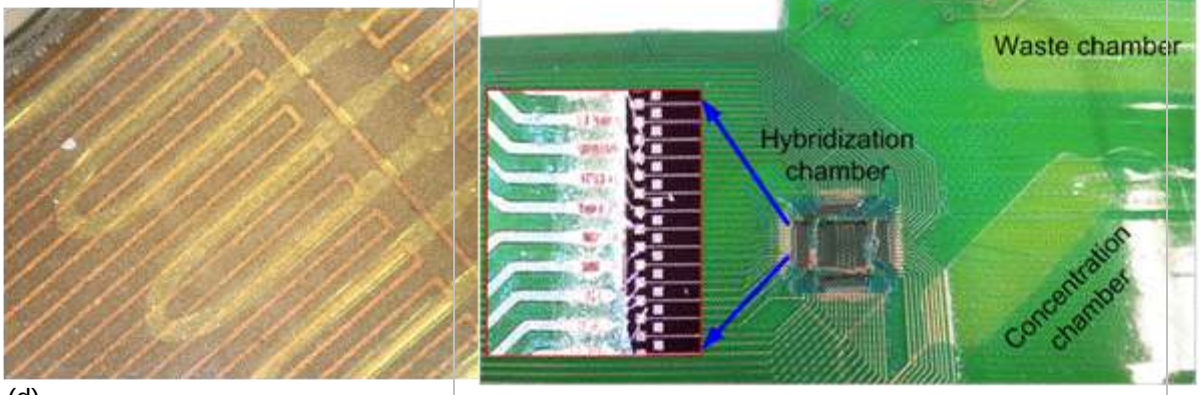
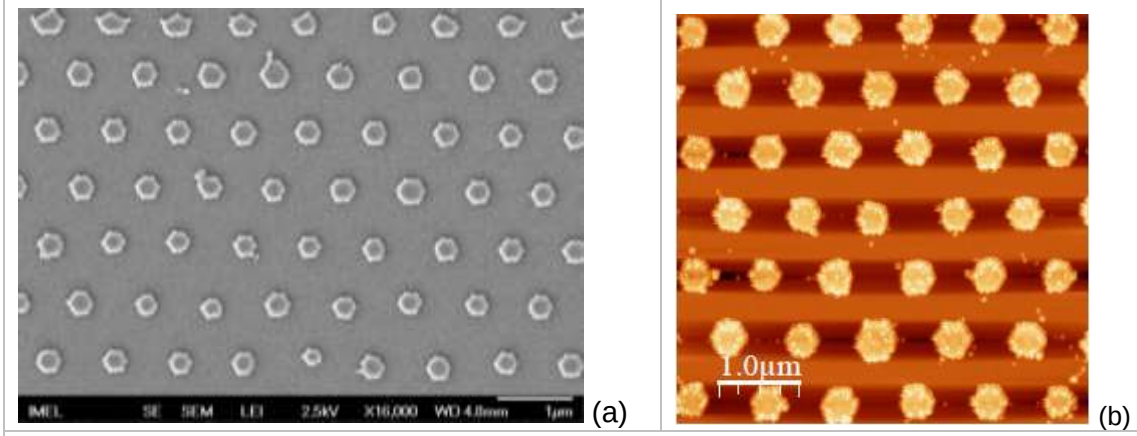


(d) (e)

Fig. 3. *(a), (b) Silicon microcolumn consisting of 32 parallel microchannels. (c) Affinity chromatography on chip is demonstrated, with retention, elution and separation of the peptide mixtures using the three different microcolumns. Some baseline shifts are due to shifting of the intensity of the Deuterium lamp. The sample quantity is 0.1nmol for each peptide. (d) Fabricated μPCR chip with integrated microheaters. (e) Prototype of a Lab-On-a-Chip on PCB integrating a μPCR device and an array of Si-based biosensors*

In the area of microarrays, a new method for nanoscale protein patterning was developed and demonstrated for very high density microarrays based on colloidal lithography and plasma processing (see Fig. 4).



(a) (b)

Fig. 4. *(a) SEM image of 300 nm SiO_2 nanoislands patterned on Si using colloidal lithography and plasma etching. (b) Atomic Force Microscopy image of SiO_2 nanoislands after selective immobilization of BSA on the plasma modified nanoislands only versus the Si surface.*

Detailed description of the above can be found in Project I.2 (Section D2)

PROJECT OUTPUT in 2012

Publications in International Refereed Journals

1. *Spectroscopic and microscopic characterization of biosensor surfaces with protein/amino-organosilane/silicon structure,*
   K.Awsiuk, A.Bernasik, M.Kitsara, A.Budkowski, P.Petrou, S.Kakabakos, S.Prauzner-Bechcicki, J.Rysz, I.Raptis
   Colloids Surf. B 90 159 (2012)

Published Conference Proceedings

1. *All-Si optoelectronic lab-on-a-chip for label-free multi-analyte biosensing*,
   I.Raptis, P.Petrou, E.Makarona, A.Botsialas, A.Psarouli, S. Kakabakos, G.Jobst, R.Stoffer, M.Hoekman, M.Sopanen, K.Tukkiniemi, K.Misiakos
   EuroPtrode 2012 (Barcelona, Spain, 04/2012)
2. *Label free biochemical determinations based on the contrast monitoring of periodic patterns*,
   I. Archontas, A. Salapatas, K. Misiakos
   IMCS 2012 , p.1359-1361, The 14th International Meeting on Chemical Sensors, May 20 - 23, 2012, Nuremberg, Germany
3. *Real-time Label-free Monitoring of Biomolecular Reactions by Monolithically Integrated Mach-Zehnder Biosensors,*
   I. Raptis, P. Petrou, E. Makarona, A. Botsialas, A. Psarouli, S. Kakabakos, G. Jobst, R. Stoffer, M. Hoekman, M. Sopanen, K. Tukkiniemi, K. Misiakos 8th Aegean Analytical Chemistry Days Conference, p. 38, September 16-20, 2012, IZTECH, Izmir, Turkey

Invited Talks

All-Silicon Optoelectronic Lab-On-a-Chip For Label-Free Multi-Analyte Biosensing: The PYTHIA Aproach,
I. Raptis, E.Makarona, A.Botsialas, K.Misiakos, P.Petrou, A.Psarouli, S.Kakabakos, G.Jobst, M.Hoekman, R.Heideman, H.Leewuis, R.Stoffer, M.Sopanen, K.Tukkiniemi
IC-MAST 2012, Budapest, Hungary, May 2012