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

EXAMPLES OF RESEARCH RESULTS IN 2010

A: Development of white light interferometric setup for label-free monitoring of biomolecular reactions.

A method was developed for label-free protein-protein binding based on the protein induced contrast modulation of the microscope image of a patterned silicon substrate. An oxidized (100 nm SiO_2) silicon wafer was patterned through photolithography and etching of the oxide in buffered HF solution, so that a chessboard pattern was created. Then, the wafer was placed in a fluidic structure consisting of two Al plates, a PDMS gasket, and plastic tubing. The top Al plate had a quartz window so that the silicon pattern could be monitored on real time while biomolecular solutions were pumped through the fluidic channels, (Fig. 1). The monitoring was performed by placing the Al plates on the microscope chuck, focusing through the quartz window on the silicon patterned surface and by filtering the microscope light in the blue (426-475 nm). Before the fluidic structure application, the wafer was silanized with APTES and coated with biotinylated Bovine Serum Albumin. Then it was dried, inserted in the fluidic structure and placed under the microscope while a micropump was supplying streptavidin solutions. The reflection coefficient of the solution-protein-oxide-silicon stack is a function of the oxide-protein combined thickness. The derivative of the reflection coefficient with respect to the protein thickness depends on the wavelength range employed and the oxide thickness. These parameters are chosen to maximize the derivative magnitude on the oxide areas. On the contrary, on the bare silicon spots where the oxide was removed, the reflection coefficient is at its maximum value and barely changes upon binding. For the parameters employed here, the oxidized areas increase their reflection coefficient upon streptavidin binding and approach the reflectivity of the bare areas. As a consequence, the contrast of the chessboard pattern drops during the experiment and the magnitude of the drop is a measure of the progress of the biomolecular reaction. After the experiment, the digital files of the pictures taken by the digital microscope camera (OLYMPUS DP71) were processed through Fast Fourier Transform to isolate the contrast factor with the highest possible resolution. The present method is the real space equivalent of the wavevector domain analysis of reflectivity by employing white light reflectometry.

Fig. 1: Aluminum plates with a light window enclosing the patterned PDMS gasket with the four independent fluidic channels. Also shown are the four fluidic inlets.

B: 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. 2a) 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.

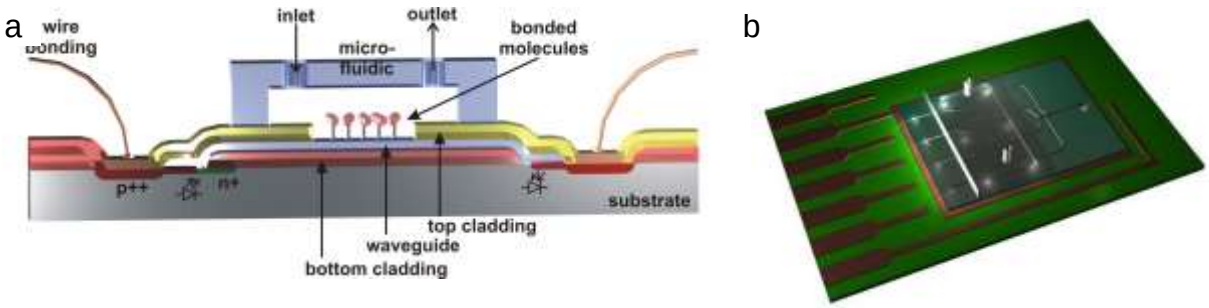


Figure 2: a) Cross section of the basic sensor concept with the integrated light source, planar waveguide (sensing arm) and photodetector. b) Schematic of the fully-integrated biosensor with an array of BB-MZI devices

The PYTHIA optoelectronic transducer is based on the monolithic optocoupler platform that has been developed at the Institute of Microelectronics and consists of waveguide coupled to a VIS-NIR light source and a photodetector. The 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. 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. Encapsulation with an appropriately designed microfluidic system 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 array will be fixed on a cartridge (fig. 2b), ready to be manually inserted to its final position in the housing, where it will be directly connected to the optical and electrical interconnects.

The PYTHIA opto-electronic transducer was realized based on the theoretical light propagation studies and fulfilling all specifications set at the beginning of the project and related to chip size, multi analyte detection capabilities and sensing performance. In particular, 4inch wafers with PYTHIA biochips hosting 10 Broad-Band Mach-Zehnder Interferometers self-aligned to ten LEDs and a common PhotoDetector were successfully fabricated. Each chip has a size of 9.26X4.00mm^2 (fig. 3) which is the **smallest multi-sensing truly integrated optical transducer ever made**. The design

was based on extensive simulations and comprised of many novel waveguide engineering features that allow for on-chip mode and polarization selection and low transmission losses. Optical evaluation of the optoelectronic transducer revealed the monomodal light propagation that is necessary for the biosensing applications targeted. Through extensive 2D and 3D optical simulation it was proved that the suggested approach is a promising sensing concept for truly integrated highly sensitive label-free optoelectronic transducers, fig. 4. In addition the simulation work revealed some design considerations that may affect the sensing performance of the suggested concept.

Fig. 3: Functionalized Fully Integrated PYTHIA chip (9.26X4.00mm^2) with the flow cell on top

First experiments with total-PSA, which is one of the targeted applications, showed the ability of the PYTHIA devices to detect analyte concentrations ranging from 5 to 100 ng/ml in serum, fig. 5.

The progress in the design and fabrication of the biochip along with the preliminary results obtained so far are convincing that the PYTHIA biochip and measuring apparatus will be able to diagnose diseases at an early stage, determine whether one will suffer from hereditary diseases and provide head-up warnings for one's well-being.

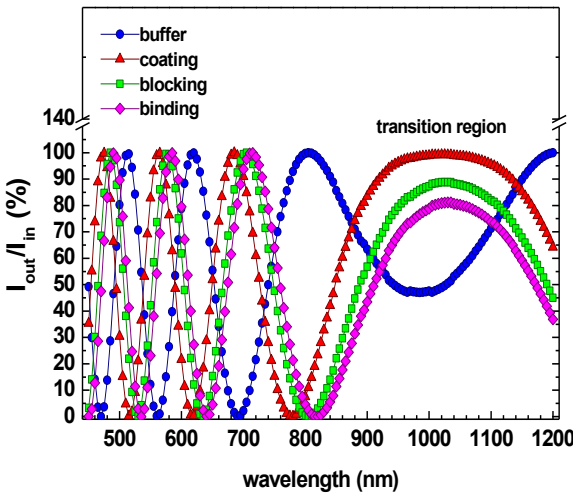


Fig. 4: Simulated TE_{00} output spectra of a BB-MZI during an immunoassay sequence. Circles: PBS buffer solution; Triangles: coating with antibody (effective adlayer thickness 4nm, n=1.45); Squares: blocking (increase in effective adlayer thickness to 5nm, n=1.45); Diamonds: binding of protein analyte (increase in effective adlayer thickness to 5nm)

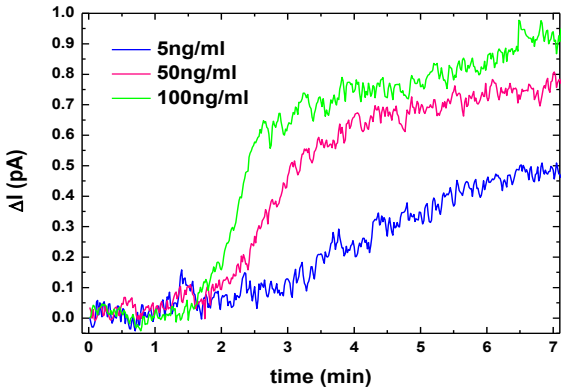
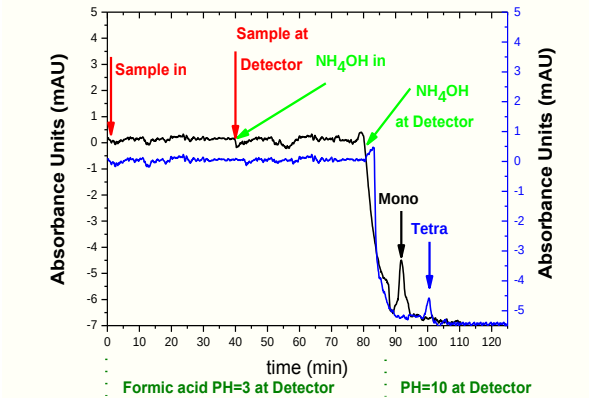


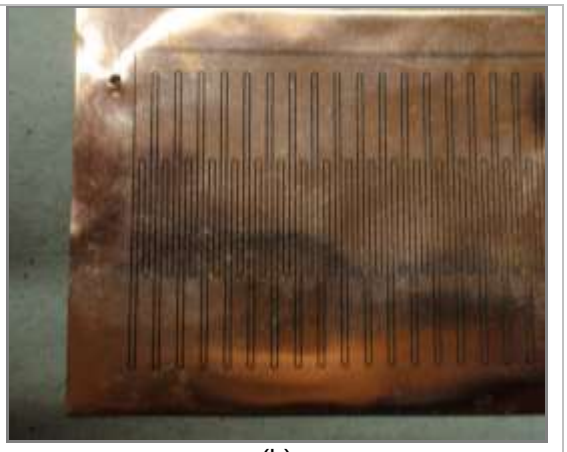
Fig. 5: Real-time signal from chips coated with a mouse monoclonal antibody against total-PSA during reaction with PSA calibrator solutions of 5, 50 and 100ng/ml in serum. Baseline was obtained by running zero calibrator.

C. Microfluidics and Microarrays

I. Deep plasma etching and bonding were implemented for the fabrication of PMMA microfluidic devices for **on-chip affinity chromatography**. Chromatography columns using TiO_2 as stationary phase were used for phosphopeptide separations (Fig. 6a).

II. A low-cost continuous flow microfluidic device (Fig.6b) with *integrated microheaters* was designed and fabricated on a thin *flexible polymeric* substrate (Pyralux polyimide, PI), in order to perform DNA amplification on chip at fast amplification rates





(b)

Fig. 6
(a) Phosphopeptide Chromatogram from a PMMA chromatographic microcolumn
(b) Part of a continuous flow PCR microfluidic device fabricated on the top side of a flexible Cu-clad PI substrate

III. A low-cost and high throughput process was demonstrated for nanoscale protein patterning on oxidized Si substrates based on colloidal lithography and plasma processing to define the spots (< 300 nm) where proteins were selectively adsorbed (Fig. 7). Such nanoscale immobilized proteins can be successfully integrated into BioMEMS and microanalytical systems.

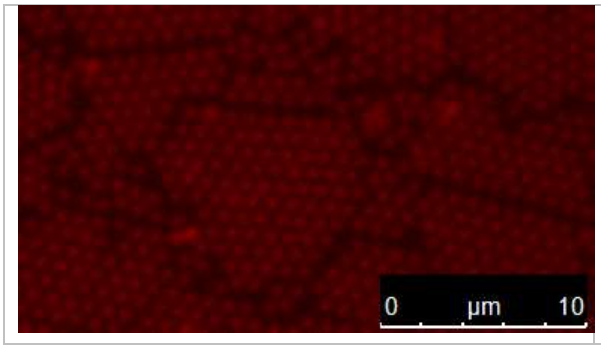


Fig. 7. Confocal Microscopy fluorescence image after selective protein immobilization on 300 nm SiO_2 (red spots) and reaction with AF-546 labeled streptavidin

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

PROJECT OUTPUT IN 2010

Publications in International Journals

1. “Regenerable flow-through affinity sensor for label–free detection of proteins and DNA” Zavali M, Petrou PS, Goustouridis D, Raptis I, Misiakos K, Kakabakos SE, J. Chromatograph. B 878, 237(2010)
2. “Biomolecular Layer Thickness Evaluation using White Light Reflectance Spectroscopy” Kitsara M, Petrou P, Kontziampasis D, Misiakos K, Makarona E, Raptis I, Beltsios K, Microelectron. Eng. 87 802(2010)
3. “Integrated optical frequency-resolved Mach-Zehnder interferometers for label-free affinity sensing” Kitsara M, Misiakos K, Raptis I, Makarona E, Optics Express 18, 8193(2010)
4. “Electrochemical biosensor microarray functionalized by means of biomolecule friendly photolithography” Mir M, Dondapati SK, Duarte MV, Chatzichristidi M, Misiakos K, Petrou P, Kakabakos SE, Argitis P, Katakis I, Biosensors & Bioelectronics Volume: 25 Issue: 9 Pages: 2115-2121, 2010
5. "Dual-cardiac marker capillary waveguide fluoroimmunosensor based on tyramide signal amplification" Niotis AE, Mastichiadis C, Petrou PS, Christofidis I, Kakabakos SE,Siafaka-Kapadai A, Misiakos K, Analytical and Bioanalytical Chemistry, Volume: 396,3, Pages: 1187-1196, 2010
6. "Fully integrated monolithic optoelectronic transducer for real-time protein and DNA detection: The NEMOSLAB approach" Misiakos K, Petrou PS, Kakabakos SE, Yannoukakos D, Contopanagos H, Knoll T, Velten T, DeFazio M, Schiavo L, Passamano M, Stamou D, Nounesis G, Biosensors & Bioelectronics, Volume 26,4, pages: 1528-1535,2010

Conference Papers

B 1. “Monolithically integrated broad-band Mach-Zehnder interferometer arrays for real-time label-free monitoring of biomolecular interactions” Kitsara M, Misiakos K, Raptis I, Stoffer R, Petrou PS, Kakabakos SE, Makarona E, EuroPtrode 2010 (Prague, Czech Republic, 03/2010)
B 2. “Characterization of ultra thin biomolecular layer stacks via white light reflectance spectroscopy” Kitsara M, Raptis I, Botsialas A, Kontziampasis D, Misiakos K, Petrou PS EuroPtrode 2010 (Prague, Czech Republic, 03/2010)
B 3. “Monolithically Integrated Biosensors based on Frequency-Resolved Mach-Zehnder Interferometers for Multi-analyte determinations” Petrou PS, Kitsara M, Makarona E, Raptis I, Kakabakos SE, Stoffer R, Jobst G, Misiakos K 32nd Annual Int. Conf. IEEE EMBS (Buenos Aires, Argentina, 09/2010)

Invited Presentations

I 1. “Monolithically integrated photonic lab-on-a-chip platform for chemical and biological applications”, Raptis I, Makarona E, Kitsara M, Petrou PS, Kakabakos S, Misiakos K Micro & Nano Engineering 2010 Conf. (Genoa, Italy, 09/2010)
I 2. “Monolithically integrated photonic lab-on-a-chip platform for biological applications”, Raptis I, Makarona E, Petrou P, Kakabakos S, Misiakos K BioPhotonics 2010 Concertation meeting (Brussels, Belgium, 11/2010)
I 3. “Lab on a chip bioanalytical devices based on silicon optocouplers” K. Misiakos, Colloquium Lectures, Department of Physics, University of Crete, December 16, 2010.

Msc Thesis

Ioannis Arhontas,Title : Label free bioanalytical determinations in real time based on the monitoring of the periodic pattern contrast on oxidized silicon, Name of supervisor K. Misiakos
Defended at SEMFE (NTUA), Master Thesi,. November 2010