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
- Develop microfluidc channels integrated on transducer silicon chips
- Use soft lithography, Deep Plasma Etching, and plasma assisted bonding to fabricate PDMS, PMMA (and other organic polymer) based microfluidic devices
- Fabricate capillary electrophoresis, and chromatography devices
- Develop open microfluidics using electrowetting actuation
- Develop novel plasma based micro array technologies

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MAIN RESULTS IN 2009

A. Bioanalytical lab-on-a-chip based on monolithic Silicon optocouplers

Monolithic silicon optocoupler array chips are properly biofunctionalized to affinity biosensors. The optocouplers consist of silicon nitride waveguides that optically link silicon LEDs and silicon photodetectors (Fig.1). The light emitters are silicon avalanche diodes that emit light when reverse biased beyond their breakdown voltage. The optical device is transformed to biosensor by spotting the waveguides with ss DNA (Fig.2) probes and by coupling a microfluidic compartment on top to allow for the supply of the reagents and the sample. Binding of the counterpart ss DNA changes the optical coupling efficiency and the detector photocurrent and allows for simultaneous detection of a variety of molecules.

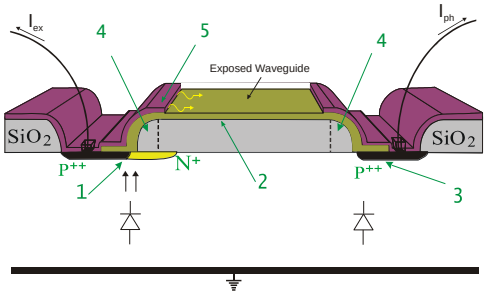


Fig. 1. *Schematic of the monolithic silicon optocoupler showing the LED on the left (1), the silicon Nitride waveguide (2) in the middle and the detector on the right (3). The thick (3 microns) silicon dioxide (4) spacers at either end of the optical link provide for the smooth fiber bending to suppress bending losses. The cladding layer (5) is removed to exposed the bioreaction waveguide surface.*

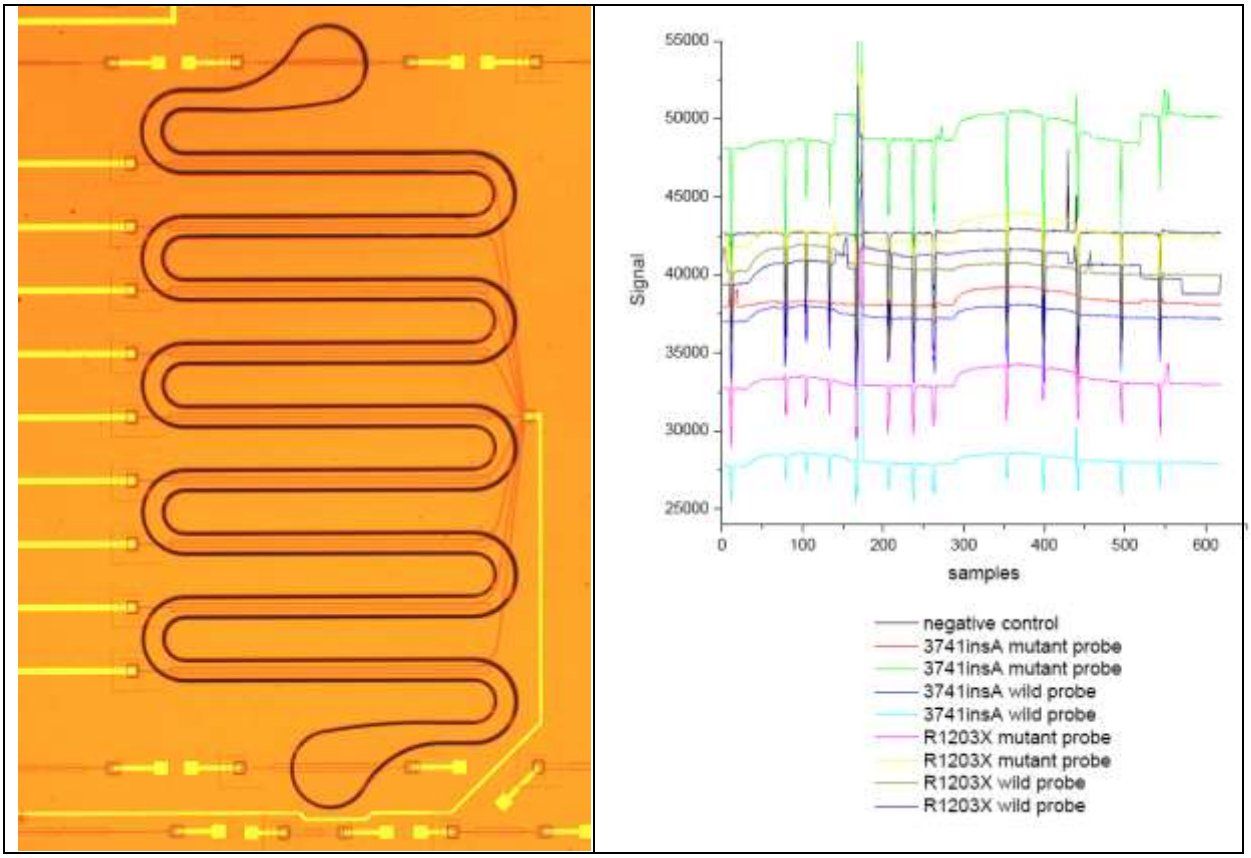


Fig. 2. View of the chip (left) and multi-analyte DNA detection sequence (right).

The chip photograph shows the nine LEDs on the left and the single detector on the right where all waveguides converge.
The middle waveguide has a length of 2400 μm, a width of 8 μm. The waveguides for most of their length are parallel with a pitch 400 μm.
Also shown is an open meandering SU-8 150 μm wide microchannel that extends over all nine waveguides and has two input-output fluidic ports
(upper-lower side). From the Al interconnect geometry it is implied that all the contact pads (not shown) are brought on the left side of the chip.
The real-time response from a chip spotted with DNA probes as indicated in the legend during hybridization reactions with PCR products.
The testing sequence was as follows (Samples=time step):
1xHEN buffer: start to sample# 25; Combined PCR products from healthy individual DNA corresponding to mutations R1203X & 3741insA:
sample# 25-100; Washing with 1xHEN: sample# 100-130; Washing with 0.5xHEN: sample# 130-160; Washing with 0.25xHEN: sample# 160-200;
Washing with 0.125xHEN: sample# 200-235; Washing with 1xHEN: sample# 235-260;
Combined PCR products from patients with the mutations R1203X & 3741insA: sample# 260-350; Washing with 1xHEN: sample# 350-400;
Washing with 0.5xHEN: sample# 400-450; Washing with 0.25xHEN: sample #450-500; Washing with 0.125xHEN: sample #500-550;
Washing with 1xHEN: sample #550-end of run. The negative spikes are due to stray light interaction following the instrument lid opening.

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 strong 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. However, MZI device presents certain drawbacks when monochromatic light is used: *Optical coupling, Ambiguity, and Signal fading.*

In the framework of the PYTHIA project, www.pythia-project.eu, a novel approach, Broad-Band Mach-Zehnder Interferometry (BB-MZI) (fig. 3a) is explored as an alternative operation principle based on a monolithically integrated biosensor array, fabricated by standard silicon technology. This

radical concept will be applied to the early diagnosis of human diseases through the label-free, multi-analyte detection of gene mutations and proteins.

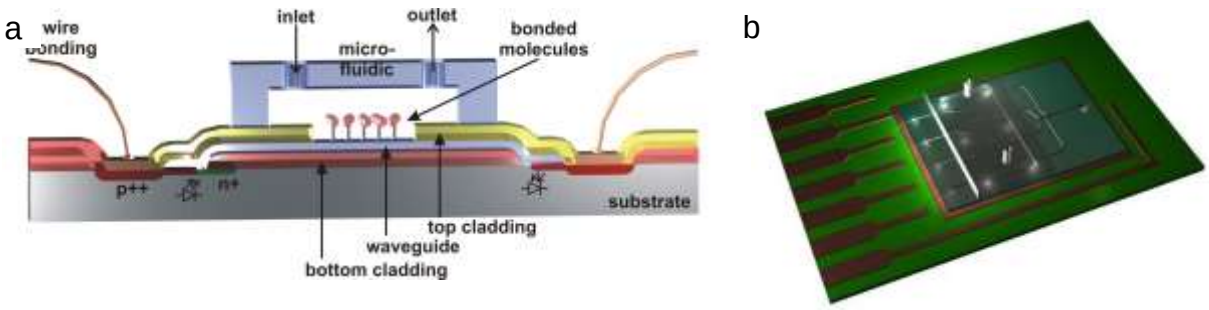


Fig. 3*: a) Cross section of the basic sensor concept with the integrated light source, planar waveguide (sensing arm) and photodetector. b) Schematic of the fully-integrated biosensor with an array of BB-MZI devices*

The basic sensor scheme consists of a VIS-NIR light source and a waveguide monolithically fabricated on a silicon wafer, while its principle of operation is the spectroscopic interference due to the optical path difference originating by biochemical events. The signal can be recorded either via an also monolithically fabricated photodetector or via an external spectrophotometer. 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 detector for multiplexing operation. Encapsulation with an appropriately designed microfluidic system will 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. 3b), ready to be manually inserted to its final position in the housing, where it will be directly connected to the optical and electrical interconnects.

Through extensive 2D and 3D optical simulation with BeamProp software (RSoft Inc.) it was proved that the suggested approach is a promising sensing concept for truly integrated highly sensitive label-free optoelectronic transducers. In addition the simulation work revealed some design considerations that may affect the sensing performance of the suggested concept. The Y junctions proved to have a significant effect on the transmitted spectrum result in a distortion away from the ideal sinusoidal shape expected from the ideal Y junctions supporting two optical paths with different lengths. Nevertheless such a distortion in no way compromises the inherently high sensitivity of the structure in detecting small changes in the effective refractive index of the exposed arm.

The first batch of un-optimized optoelectronic transducer arrays was fabricated and evaluated. The electrical and optical characterization of these optoelectronic transducers provided a solid confirmation of the BB-MZI concept and useful information for the design optimization of the biosensor chips. The transducers were functionalized and encapsulated with a microfluidic cell to be possible to evaluate them in real conditions. For the first evaluation of the PYTHIA device performance, model assays were used. For the Biotin-Streptavidin model assay, the detection of streptavidin in the picomolar range was easily demonstrated, which is comparable with the state of the art values for label-free biosensors. These preliminary results are promising for ultra sensitive detection of proteins and DNA mutations.

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.

C. Microfluidics and Microarrays

For microfluidics, we use Deep Plasma Etching and bonding to fabricate PDMS, PMMA, PEEK and Si microfluidic devices. We fabricated on chip affinity chromatography columns using TiO_2 as stationary phase for phosphopeptide separations. We also demonstrated "smart" microfluidics incorporating capillary pumping and hydrophobic valving using our "plasma toolbox" technology (see Fig. 4a).

In addition using our plasma processes for stochastic nano-texturing of polymers, we demonstrated high density protein microarrays both on PDMS and on PMMA, with almost 10fold higher binding capacity of proteins, and 100fold increased sensitivity compared to flat substrates. We have also extended self aligned processes for protein microarrays on prepatterned surfaces to cheaper substrates (see Fig. 4b).

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

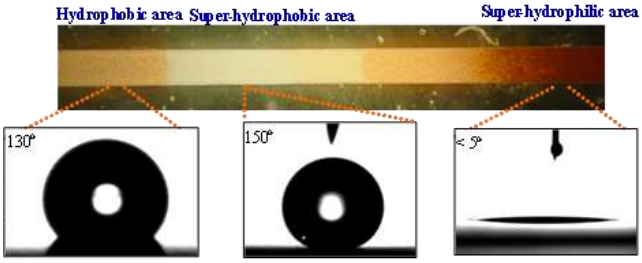
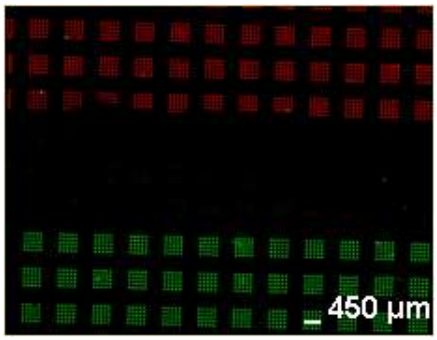


Fig. 4.

Three types of surfaces in a microchannel with different wetting properties (hydrophobic, superhydrophobic and super hydrophilic) in the microchannel inner surface. The superhydrophobic stripe functions as a passive valve preventing capillary pumping of fluid from one superhydrophilic area to another

(a)



Immobilization of b-BSA (red) and RgG (green) model proteins on SU8 spots (50µm) defined through lithography, after SF_6 plasma treatment of the glass substrate

(b)

PROJECT OUTPUT in 2009

Publications in International Journals

1. "Real-time label-free detection of complement activation products in human serum by white light reflectance spectroscopy", Petrou PS, Ricklin D, Zavali M, Raptis I, Kakabakos SE , Misiakos K, Lambris JD, Biosensors & Bioelectronics, 24, p.3359-3364, 2009
2. "Ultra-thin poly(dimethylsiloxane) film-coated glass capillaries for fluoroimmunosensing applications", Niotis AE, Mastichiadis C, Petrou PS, Siafaka-Kapadai A , Christofidis I , Misiakos K, Kakabakos SE, Microelectronic Engineering, 86 p. 1491-1494, 2009
3. "Bulk fluorescence light blockers to improve homogeneous detection in capillary-waveguide fluoroimmunosensors", Mastichiadis C, Petrou PS, Christofidis I, Misiakos, Kakabakos SE, Biosensors & Bioelectronics,24,p.2735-2739,2009
4. "A monolithic photonic microcantilever device for in situ monitoring of volatile compounds", Misiakos K, Raptis I, Gerardino A, Contopanagos H. Kitsara M, LAB ON A CHIP,9, p.1261-1266, 2009
5. "Real-time detection of BRCA1 gene mutations using a monolithic silicon optocoupler array", Mavrogiannopoulou E, Petrou PS, Kakabakos SE Misiakos K, Biosensors & Bioelectronics, 24,p. 1341-1347, 2009
6. "Silicon optocouplers for biosensing", Petrou PS, Kakabakos SE, Misiakos K, International Journal Of Nanotechnology, 6, p.: 4-17 Published: 2009
7. "Capillary waveguide fluoroimmunosensor with improved repeatability and detection sensitivity", Niotis AE, Mastichiadis C, Petrou PS, Christofidis I,Siafaka-Kapadai A, Misiakos K, Kakabakos SE, Analytical And Bioanalytical Chemistry,393 p.1081-1086, 2009
8. "A low temperature surface modification assisted method for bonding plastic substrates", Vlachopoulou ME, Tserepi A, Pavli P, Argitis P. Sanopoulou M. Misiakos K., Journal Of Micromechanics And Microengineering, 19, A.N.: 015007, 2009

Publications in International Conference Proceedings

1. "Ultra-miniaturized monolithically integrated polymer coated Si optoelectronic cantilevers for gas sensing applications", Misiakos K, Raptis I, Goustouridis D, Gerardino A., Contopanagos H, Valamontes E, Kitsara M, IEEE Sensors 2009 (Christchurch, New Zealand, 10/2009)
2. "A flow-through optical sensor system for label-free detection of proteins and DNA", Petrou P.S, Zavali M, Raptis I, Beltsios K,.Kakabakos S.E, Ricklin D, Lambris J.D, Misiakos K, IEEE Sensors 2009 (Christchurch, New Zealand, 10/2009)

Conference Presentations

1. "Evaluation of biomolecular film thickness using white light reflectance spectroscopy"Kitsara M, Petrou P, Beltsios K, Raptis I, Kakabakos S, Instrumental Methods of Analysis (IMA 2009) (Athens, Greece, 10/2009).