PROJECT IV: BIO-MICROSYSTEMS

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Objectives:

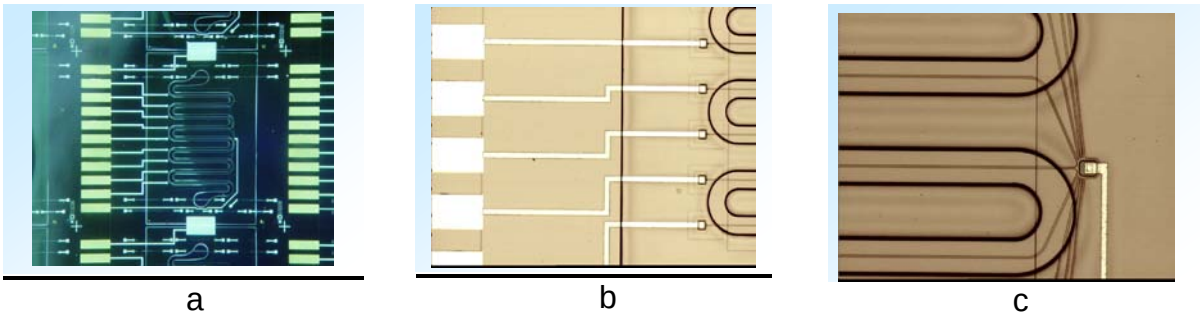
- Development of bioanalytical lab-on-a-chip devices based on monolithic optoelectronic transducers (bioactivated optocouplers).
- Development of monolithically integrated interferometric biochips for label-free biosensing
- Development of white light interferometric setup for label free monitoring of 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

Funding:

- EU, IST, STREP, "NEMOSLAB", NanoEngineered Monolithic Optoelectronic transducers for highly Sensitive and LAbel-free Biosensing (coordinated by K. Misiakos start 1-1-2006, end 30-6-2009)
- EU, FP7-ICT, STREP, "PYTHIA", Monolithically integrated interferometric biochips for label-free early detection of human diseases (coordinated by I. Raptis start 01-05-2008, duration 36months)

EXAMPLES OF RESEARCH RESULTS IN 2008

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



a b c

Figure 1**.** Photograph of a completed optocoupler chip showing gold pad contacts, Al interconnects, especially the detector long interconnect to the opposite side (single sided geometry) (b). Also shown are SU-8 open microchannels, the LEDs (b) and the single detector where all waveguides converge (c)

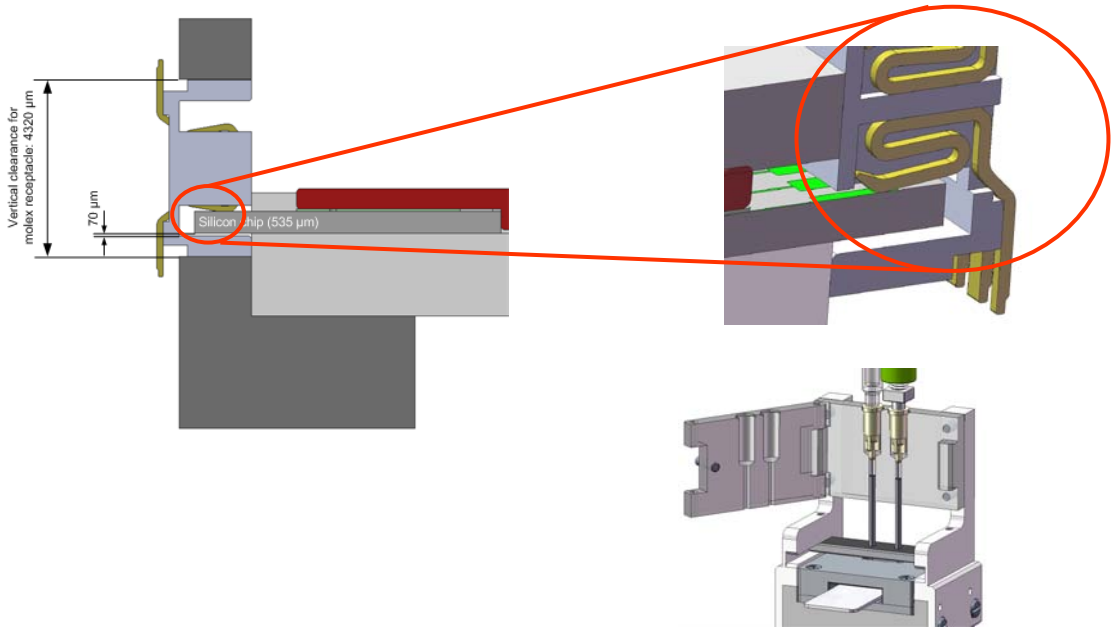


Figure 2. Packaging and testing of the optocoupler chips. The chips is packaged in a cartidge (left) and insertred in a socket where a board-to-board connector is making contact with the pads. The sample and reagents for the bioassay are introduced via syringes coming from above (lower right).

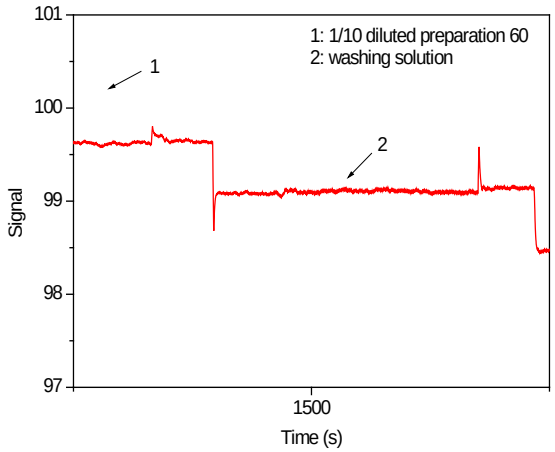


Figure 3. Real-time response from a 2 micron wide and 150 nm thin silicon nitride waveguide coated with streptavidin during flow of vesicles at a 1/10 dilution in 80 mM NaCl, 10 mM phosphate buffer, pH 7.4. The flow rate was 100 µl/min. The vesicles had a diameter of 100 nm, had biotin molecules on their outer surface and were containing a fluor with absorption at 650 nm. Individual binding and de-binding events are evident.

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.*

We are suggesting a novel approach, **Broad-Band Mach-Zehnder Interferometry (BB-MZI)** (fig. 1), as an alternative operation principle for optical biochemical sensors that can form the basis of versatile and **ultra-sensitive label-free, multi-analyte detection schemes**. The characteristics of the suggested method were compared with the ones from the conventional Single-Wavelength MZI (SW-MZI) structure.

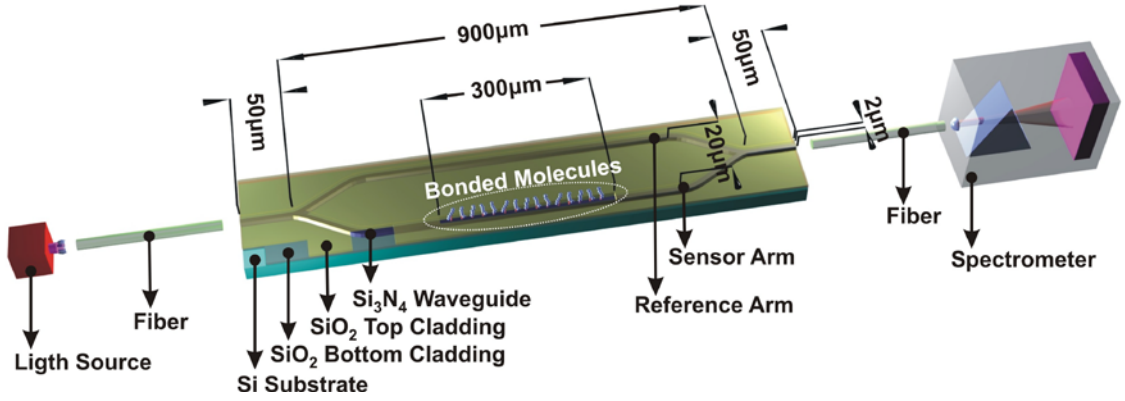


Figure 1: Broad-Band Mach-Zehnder Interferometry Approach

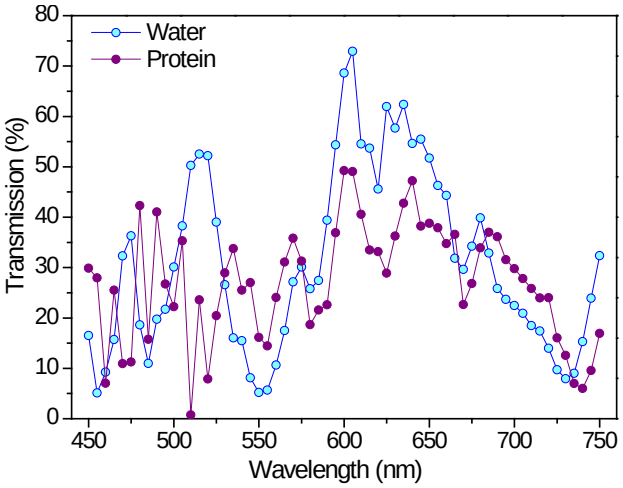


Figure 2: Transmission Spectra of the 1mm long BB-MZI for pure water and 3nm thick protein adlayer (n=1.38) bound on the sensing arm

To evaluate the BB-MZI performance, 2D and 3D optical simulation with BeamProp software was carried out by considering aqueous glucose solutions (model system) in the sensing area and protein adlayers (biosensing) as well. The simulation considerations were: 1mm overall BB-MZI length, incoherent light as input, human γ-immunoglobulin, IgG, on the sensing arm. In fig. 2 the simulation results for the biosensing case are illustrated for the 450-750nm spectrum. The ultra-thin protein adlayer can be detected by the proposed BB-MZI, either by recording the spectrum changes (if a spectrometer is employed) or by the changes of the integrated intensity (if a photodetector is opted as the recording medium).

To evaluate the BB-MZI approach, 1mm long structures were designed by using the simulation results and fabricated (fig. 3). The bottom (3μm thick) and top cladding (2μm) layers are from SiO_2 and the core from Si_3N_4. The fabrication of MZI structures was carried out with 2.0μm conventional silicon processing technologies. In fig. 4 the optical efficiency of the fabricated BB-MZI structures for various geometries is illustrated along with results from simulation. A very good agreement is shown between the simulation and experimental results. Furthermore, a very good repeatability is monitored between the structures fabricated in different Si dies.

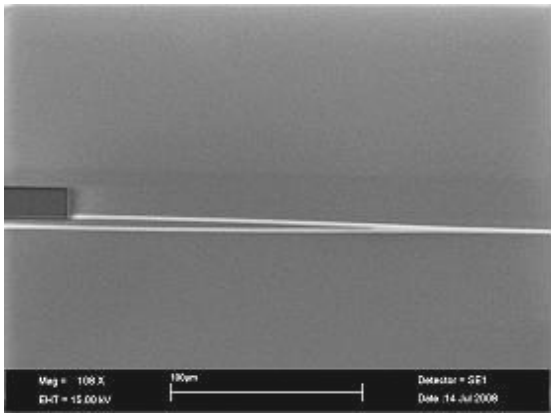


Figure 3: top-down SEM image of the sensing arm are of the BB-MZI test structures

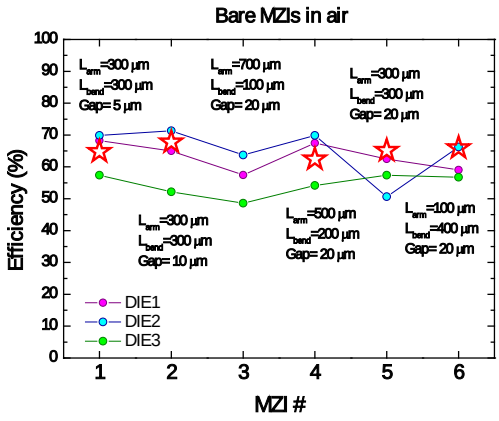


Figure 4: BB-MZI output efficiency for various geometries

C. Microfluidics and Microarrays

Objectives:

- For microfluidics we use Deep Plasma Etching, and plasma assisted bonding to fabricate PDMS, PMMA, PEEK and Si microfluidic devices, such as chromatography columns. We also demonstrate a novel plasma-based protein patterning process for micro array fabrication.
- We nano-texture polymers in plasmas, and find that protein adsorption is greatly enhanced on such smart surfaces.

1. Fabrication of microfluidic devices on plastic substrates and Silicon using deep plasma etching

1.1 Plasma etching of PMMA, PEEK, PDMS, and Silicon microfluidics

(K. Tsougeni, K. Kontakis, N. Vourdas, M. Vlachopoulou, G. Boulousis, A. Tserepi, E. Gogolides)

We demonstrate a new mass production amenable technology for fabrication and surface modification of plastic disposable microfluidic devices, namely direct lithography on the plastic substrate followed by deep polymer etching. We applied plasma processing to fabricate polymeric microfluidics in Poly(methyl methacrylate) (PMMA) and Poly(ether ether ketone) (PEEK), PDMS, and Silicon. Deep anisotropic O_2 or SF6 or SF6/ C4F8 plasma etching was utilized to etch (pattern) the polymeric substrate after lithography. Etch rates were optimized to minimize the process time and surface roughness was controllably adjusted from very rough (high aspect ratio nanocolumns) to smooth channels, by choosing appropriate plasma conditions. *We demonstrated control on the topography of the etched surfaces, depending on the etching conditions; either smooth surfaces or very rough columnar-like surfaces were obtained.* After engraving the PMMA, PEEK and Si microfluidic a bonding step was done to seal the channels and provide their fourth wall. Fig. 1a demonstrates a PEEK plate, after the plasma treatment and after sealing with a pressure adhesive. Demonstration of a mixer was also done on PMMA (Fig. 1b). For PDMS oxygen plasma bonding was done.

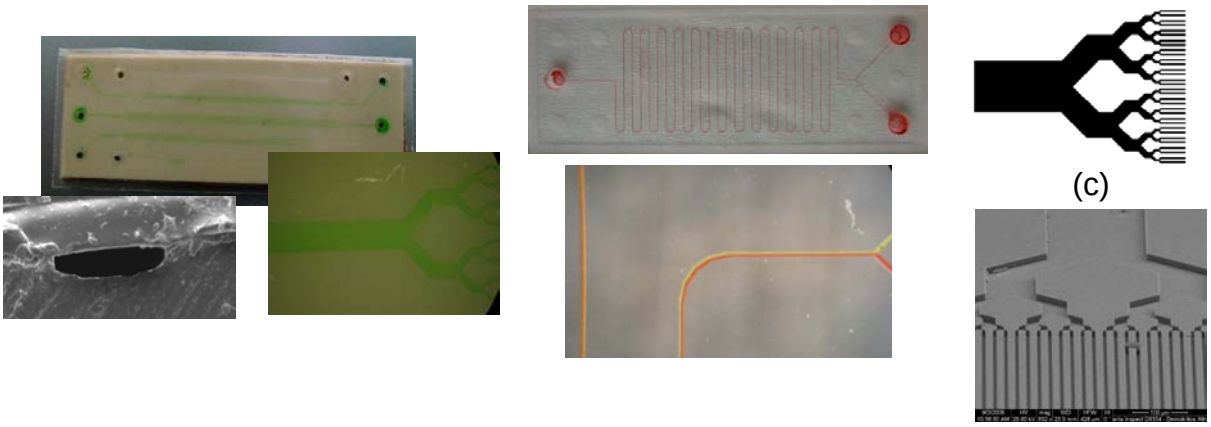


Fig. 1: (a) The microfluidic channel of PEEK and details in SEM of the cross section after the sealing. (b) Mixing of two liquids in PMMA plasma etched microfluidic mixer. (c), (d) Photos of the mask layout of parallel channels in Silicon microfluidic devices.

2. Protein microarrays on plasma-treated substrates and protein adsorption on plasma nanotextured polymers

2.1 Method for fabrication of protein microarrays through plasma treatment of patterned substrates

(P. Bayiati, A. Malainou, A. Tserepi, P. S. Petrou, S. E. Kakabakos)

Protein patterning through plasma selective FC deposition on patterned SiO_2/Si and glass substrates is pursued. The capability to immobilize two different proteins on such substrates was demonstrated (Fig. 2(a)), while the stability of protein binding on C_4F_8 plasma treated surfaces was also investigated and was found comparable to commercial PS microtitration plates. Therefore, with the proposed method, high density and high quality (signal to noise 25:1, Fig. 2(b)) protein microarrays can be fabricated exhibiting very good intra-spot homogeneity and inter-spot repeatability.

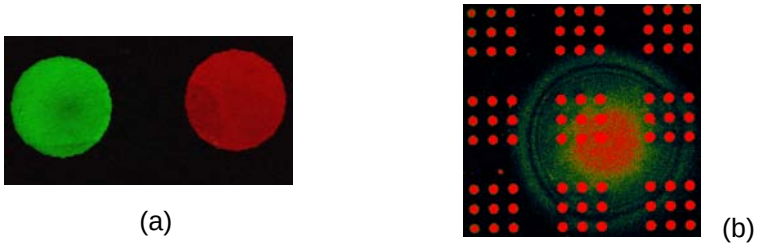


Fig. 2: (a) Fluorescence image of fluorocarbon modified Si substrate bearing SiO_2 spots after immobilization of two different proteins, gamma globulin IgG (green spot) and b-BSA (red spot), (b) fluorescence image of a modified glass substrate patterned with AZ photoresist, demonstrating selective (10:1) protein adsorption on 100 μm glass spots after treatment in O_2 plasmas

2.2 Plasma nanotexturing of PMMA and PDMS for increased protein adsorption

(K. Tsougeni, M. Vlachopoulou, P. S. Petrou, S. E. Kakabakos, E. Gogolides)

We demonstrated fabrication of random columnar/filamented-like, low and high-aspect ratio micro or nano-structures based on O_2 plasma-induced roughening (nanotexturing) of poly(methyl methacrylate) (PMMA), or SF6 roughening of PDMS. The effect of topography and protein adsorption capacity was investigated. Conditions (plasma treatment, ageing) are sought for maximum and

uniform protein adsorption on nanotextured PMMA surfaces. Specifically, adsorption of biotinylated-BSA was found to increase with plasma duration. A 2x-4x times increase in protein adsorption (depending on the protein concentration) was observed following 5-60 min plasma treatment compared to untreated surfaces. Highly homogeneous bright protein microspots on such optimized plasma-nanostructured surfaces are also shown.

(a) (b) (c) (d)

Fig. 3: Fluorescence images of b-BSA spots on a (a) flat untreated, (b) 5-min 20-min O_2 plasma-treated PMMA surface. Spotting of b-BSA on fresh SF_6 treated surfaces with a nanoplotter. Fluorescence images of spots of 200 μg/ml b-BSA (c) on an untreated PDMS surface, (d) on a 6 min SF_6 treated PDMS surface.

More details of the above can be found in Project I.2 Lithography and Plasma Processes for Electronics, Microfluidics, and Surface Nano-Engineering.

PROJECT OUTPUT in 2008

Publications in International Journals

1. "Monolithic silicon optocoupler engineering based on tapered waveguides" K. Misiakos, E. Makarona, M. Kitsara, I. Raptis, Microelectronic Engineering, 85, 1074-1076, (2008)

Conference Papers

1. "Integrated biochemical broad-band Mach-Zehnder sensors", M.Kitsara, I.Raptis, K.Misiakos, E.Makarona EuroSensors 2008 conference, Dresden, Germany, 09/2008
2. "Broad-band Mach-Zehnder Interferometry as a detection principle for label-free biochemical sensing", M.Kitsara, I.Raptis, K.Misiakos, E.Makarona, IEEE Sensors 2008, Lecce, Italy, 10/2008)

For publications concerning **Microfluidics and Microarrays:**
see relevant publications of **Project I.2** Lithography and Plasma Processes for Electronics, Microfluidics, and Surface Nano-Engineering.

Patent Applications

1. "Monolithically integrated physical chemical and biological sensor arrays based on broad-band Mach-Zehnder Interferometry" (inventors, I. Raptis, E. Makarona, M. Kitsara, K. Misiakos, S. Kakabakos, P. Petrou, OBI Application 20080100174)
2. "Integrated optoelectronic silicon biosensor for the detection of biomolecules labeled with chromophore groups or nanoparticles" (inventors K. Misiakos, S. kakabakos, I. Raptis, E. Makarona, P. Petrou, OBI application 20080100390)

Organization of Workshops

The 3rd International Workshop on Multianalyte Biosensing Devices, was organized in Athens, September 18-19, 2008. The workshop was attended by 30 participants. Detailed information about the scope and the program of the workshop can be found at http://www.imel.demokritos.gr/projects/nemoslab/workshop/workshop.htm