Project III. 3: BIO-MICROSYSTEMS

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Research orientation:

- Development of bioanalytical lab-on-a-chip devices based on monolithic optoelectronic transducers.

Main results in 2005:

The main results obtained in 2005 within the different tasks of the project are given below.

Task 1 Multi-protein and DNA assay using chromophore labels

a) Protein Coating

The monolithic silicon micro-optical transducer employed in these experiments is shown in fig.III.3.1 and III.3.2. Coating was performed by applying on three different waveguides on the same chip solutions of biotinylated bovine serum albumin (BSA), mouse IgG and rabbit IgG solutions (20 mg/L in 0.05 M carbonate buffer, pH 9.2), respectively, using a microsyringe dispenser aligned on top of the optocouplers of the pretreated wafer. The wafer was placed on a x-y motorized stage under a microscope for monitoring the spotting process. The spotting solutions were incubated for 30 min at room temperature (RT). The chips were then washed with distilled water and blocked with a 10 g/L BSA solution in 0.1 M $NaHCO_3$ buffer, pH 8.5, for 30 min at RT. After washing as previously, the surface was dried under a nitrogen stream. The results of the assay on the chip spotted with the three different proteins are shown in fig. III.3.3 in real time. The anti-mouse IgG antibody, streptavidin (2 nM) and anti-rabbit IgG antibody were sequentially pumped through the fluidic system. Here, the R-phycoerythrin fluorescent labels are used as chromophore groups to absorb photons from the waveguided modes through evanescent wave interactions. The responses shown in fig. III.3.3 have the same starting point for every curve by shifting the injection moments of the respective protein in the fluidic cover. As expected, and due to the higher binding constant, the response to streptavidin of the optocoupler coated with biotinylated BSA was stronger compared to the other two despite the fact of the lower streptavidin concentration. The optocouplers coated with mouse IgG and rabbit IgG showed responces roughly proportional to the anti-mouse IgG antibody and anti-rabbit IgG antibody concentrations, respectively.

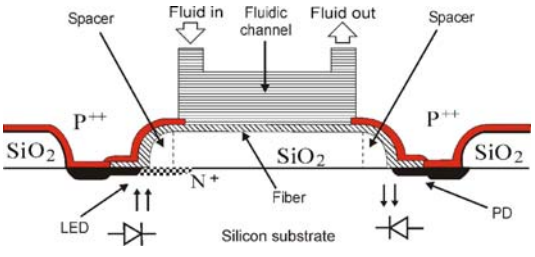

Fig.III.3.1: *Schematic Illustration of the monolithic silicon optocoupler biosensor coupled to the fluidic module*

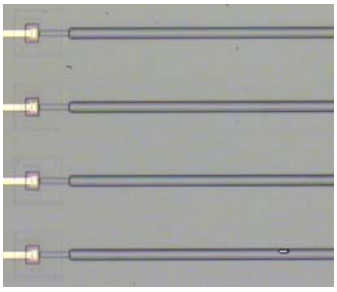

Fig.III.3.2: *Photograph of an array of optocouplers with the emitters on the left and the fiber in the middle (detectors not shown)*

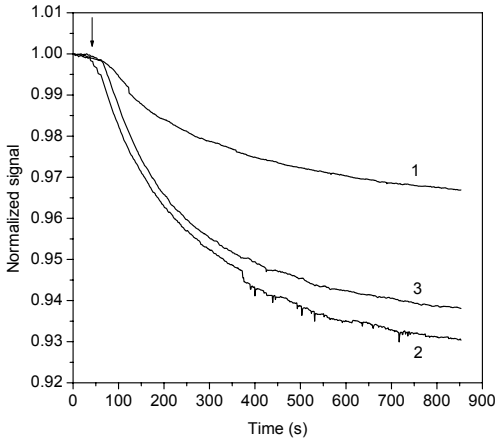

Fig. III.3.3: *Real time signal evolution obtained from three different fibers coated with mouse IgG (1), biotinylated BSA (2) and rabbit IgG (3) upon reaction with R-phycoerythrin labeled anti-mouse IgG antibody (5 nM), streptavidin (2 nM) and anti-rabbit IgG antibody (10 nM), respectively. Initial baseline was obtained by pumping through the fluidic only the assay buffer. The arrow indicates the injection of the respective labeled reagents. The signals are normalized with respect to their initial value.*

b) DNA coating

The waveguide surface was coated with a 20 mg/L biotinylated BSA solution in 0.05 M carbonate buffer, pH 9.2, for 30 min at room temperature (RT). The chips were then washed with distilled water and blocked with a 10 g/L BSA solution in 0.1 M $NaHCO_3$ buffer, pH 8.5, for 30 min at RT. After washing as previously, the surface was dried under a nitrogen stream. A biotinylated oligonucleotide (0.5 µM), corresponding to the mutant sequence of the 3099delT mutation (biotin-5'-AAACTAAAGTAAGAAAAA-3') in the BRCA1 gene, was pre-incubated with streptavidin (0.166 µM) for 30 min at a 0.05 M phosphate buffer, pH 6.5. This solution was then applied onto chips covered with biotinylated BSA and incubated for 30 min at RT. The chips were then washed with distilled water and dried under nitrogen stream. For the hybridization assay, the AlexaFluor 546 fluorophore was employed as the chromophore group. The fully complementary oligonucleotide clearly shows a photocurrent drop (fig. III.3.4) which is absent in the non-complementary oligonucleotide, and points out a good selectivity between the mutant and the wild type sequence.

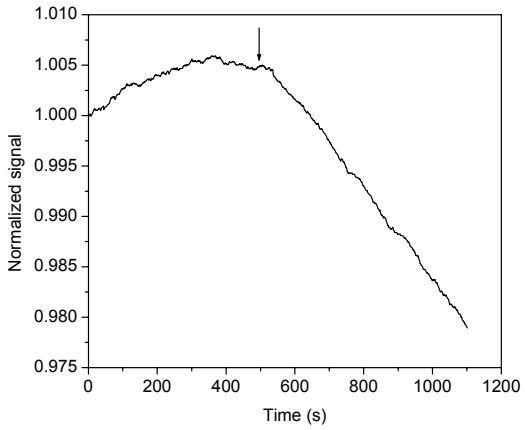

Fig. III.3.4: *DNA hybridization assay. Signal obtained in real time by pumping through the fluidics module a solution of the non-complementary oligonucleotide for approximately 500 seconds followed by injection (arrow) of the fully complementary oligonucleotide. Both oligonucleotides were labeled with AlexaFluor 546.and were used at a concentration of 0.1 µM. The signal is normalized with respect to its initial value.*

From fig. III.3.3 and III.3.4 we conclude that commercially available fluorophore groups can be employed as effective chromophores and provide bioanalytical results with the presented transducer. The selection of the particular fluors, R-phycoerythrin with AlexaFluor 546, was based on their absorption spectrum which matches, to some extent, the emission spectrum of the avalanch LEDS. Other labels with absorption peaks in the 530nm-670 nm spectral region could also be employed.

Task 2 Label-free protein assay

Here, the optocouplers were coated with biotinylated BSA so that colloidal gold-labeled streptavidin could selectively bind and give an initial photocurrent drop due to the nanoparticle plasmonic response. The amount of photocurrent drop is an increasing function of the effective refractive index of the medium that surrounds the gold nanoparticles. Consequently, if a biotinylated protein reacts with the immobilized streptaviding the gold nanoparticles will sense a slightly different medium in their vicinity which will result in a different photocurrent drop. This way the gold-labeled streptavidin is used as a capture molecule which at the same time exhibits a photon extinction cross section that depends on the molecules bound on the nanoparticle. Therefore, label free detection is possible of biotinylated biomolecules. Such a label free assay where biotinylated mouse IgG was employed as the analyte is shown in fig. III.3.5. Upon injection of the protein, the photocurrent starts dropping for about 200 seconds and then reaches a new baseline. It should be noted that this reaction proceeds at a much faster speed compared to the rates observed in fig. III.3.3. Such a trend could be attributed to the label free nature of the reaction in fig. III.3.5.

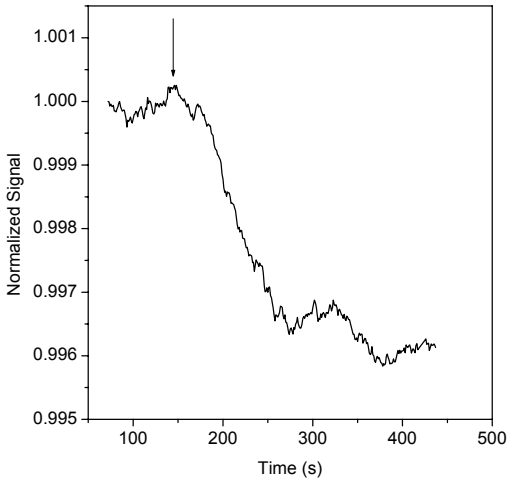

Fig. III.3.5: *Label-free protein assay. Real time signal obtained from an optocoupler modified with streptavidin-gold nanoparticles conjugate. Initial baseline was obtained by pumping assay buffer (PBS) for 150 seconds with a flow rate of 20 μL/min prior to injection of biotinylated mouse IgG solution in 0.05 M PBS, pH 7.4, at a concentration of 1 nM.*

PROJECT OUTPUT in 2005

PUBLICATIONS in INTERNATIONAL JOURNALS and REVIEWS

1. "157-nm Laser ablation of polymeric layers for fabrication of biomolecule microarrays", Douvas, A.M., Petrou, P.S., Kakabakos, S.E., Misiakos, K., Argitis, P., Sarantopoulou, E., Kollia, Z., Cefalas, A.C., Analytical and Bioanalytical Chemistry 381 (5), pp. 1027-1032
2. "Self assembled structures on fluoro-polymers induced with laser light at 157 nm", Kollia, Z., Sarantopoulou, E., Cefalas, A.C., Kobe, S., Argitis, P., Misiakos, K., A*pplied Surface Science* 248 (1-4), pp. 248-253
3. "Patterning of thick polymeric substrates for the fabrication of microfluidic devices", Vlachopoulou, M.E., Tserepi, A., Vourdas, N., Gogolides, E., Misiakos, K., Journal of Physics: Conference Series 10 (1), pp. 293-296
4. "A bioanalytical microsystem for protein and DNA sensing based on a monolithic silicon optoelectronic transducer", Misiakos, K., Petrou, P.S., Kakabakos, S.E., Ruf, H.H., Ehrentreich-Forster, E., Bier, F.F., Journal of Physics: Conference Series 10 (1), pp. 273-276

CONFERENCE PRESENTATIONS

1. "Biochip-compatible packaging and microfluidics for a silicon optoelectronic biosensor", H.H. Ruf, T. Knoll, K. Misiakos, R.B., Haupt, M. Denninger, L.B. Larsen, P.S. Petrou, S.E. Kakabakos, E. Ehrentreich,-Foerster, F.F. Bier, 31st International Conference on Micro- and Nano-Engineering 2005, 19-22 September 2005, Vienna, Austria. Book of Abstracts MNE-2005 ID 00444 3-o_05.
2. "Monolithic silicon optoelectronic tranducers and elastomeric fluidic modules for bio-spotting and bio-assay experiments", K. Misiakos, P.S. Petrou, S.E. Kakabakos, M.E. Vlahopoulou, A. Tserepi, E. Gogolides, H.H. Ruf, 31st International Conference on Micro- and Nano-Engineering 2005, 19-22 September 2005, Vienna, Austria. Book of Abstracts MNE-2005 ID 00492 8A_02.

PhD DISSERTATION

1. "Simulation – design and fabrication of rear point contact silicon solar cells", K. Kotsovos, Department of Electrical and Computer Engineering, NTUA, June 2005