Advances in Optical Biosensing Technologies

BLOCH ELECTROMAGNETIC SURFACE WAVE
BIOSENSORS FOR EARLY CANCER DIAGNOSIS

Open Workshop of the EU funded BILOBA Project

Rome, September 23, 2015

SAPIENZA Università di Roma, Roma, Italy
Civil and Industrial Engineering Faculty
San Pietro in Vincoli

PROGRAM

Topics and Scope

Advances in Optical Biosensing Technologies is a one-day workshop focused on the latest advances and applications of optical biosensing technologies in life sciences. It aims at bringing together the technology inventors and providers and the end-users. The open workshop is one of the communication and dissemination activities of the BILOBA Project.
The meeting will review the considerable progress in the field and provide information on the most practical emerging techniques. This is a chance for optical sensing techniques users everywhere to glimpse the latest applications and to help shape the future of research in their field.

It is a LightTalk - The Power of Photonics

The BILOBA Workshop is one of the *LightTalks - The Power of Photonics* organized by the LIGHT2015 and GoPhoton projects during the weekend of Light (September 25-28, 2015) to promote the importance of photonics to young people, entrepreneurs and the general public in Europe during the International Year of Light.

Organizing Committee

Workshop Chairman	Francesco Michelotti, SAPIENZA Università di Roma
Local Committee	Alberto Sinibaldi Oleksiy Anopchenko Agostino Occhicone
Program Committee	Norbert Danz, Fraunhofer Institute IOF, Germany Emmanuel Maillart, HORIBA Jobin Yvon, France Chiraz Frydman, HORIBA Jobin Yvon, France Federico Bussolino, Università degli Studi di Torino

INVITED TALKS

Welcome and Introduction 9:00 - 9:30

Francesco Michelotti
SAPIENZA Università di Roma
Roma, Italy

Morning Session 1 – Chair: F. Michelotti 9:30 – 11:00

Nanophotonics for Biosensing 9:30 – 10:00

Romain Quidant
ICFO - The Institute of Photonic Sciences
Barcelona, Spain

Two decades of extensive research in the field of nanophotonics have shown that resonant optical nanostructures stand as ideal systems to extend light control well beyond the limit of diffraction. These unique optical properties offer new opportunities in the field of biotechnologies and in particular show great promises for the next generations of integrated biosensors with unprecedented performances. In this talk we first review recent developments in the field of nano-optics for biosensing before presenting our own work focused on nanoplasmonics. We present an integrated analytical platform that combines the sensing capability of plasmonic antennas with advanced microfluidic technologies for the label-free multiplexed detection of protein cancer markers in blood. Our platform enables parallel, real-time detection of several markers from a single drop of human serum with a sub-ng/ml sensitivity and is currently tested for early diagnosis and treatment monitoring. We also discuss how this technology can be extended to on-a-chip chiral sensing capable to detect the handiness of biomolecules.

Surface Plasmon Resonance Based Biosensing 10:00 – 10:30

Emmanuel Maillart
HORIBA Jobin Yvon Europe Research Centre
Palaiseau, France

Since 1983 and the publication of the first application of SPR to biomolecule detection, the interest for this label-free measurement technique never ceased to increase: new ideas on SPR based measurement principle, instrumental configurations and biosensing applications are published or patented with an exponential growth. In this talk, some of the various SPR measurement hardware configurations will be presented but also a description of all the non-optical but still fundamental aspects needed for a good biosensing measurement (software, electronics, microfluidics, thermics, surface chemistry and biology). Since parallel detection of multiple biomolecules is often a requirement in biosensing, different solution for multiplex SPR detection will be presented. The following part will be focused on the very broad spectrum of possible applications. Mention will be made on some of the possible measurements coupling like MS, Raman or Fluorescence. Future trends of SPR based biosensing will be reported.

BILOBA Bloch Electromagnetic Surface Wave Biosensors for Early Cancer Diagnosis 10:30 – 11:00

Norbert Danz
BILOBA Consortium and Fraunhofer Institute for Applied Optics and Precision Engineering
Jena, Germany

The results obtained in the EU funded STREP project *BILOBA- Bloch electromagnetic surface wave bio-sensors for early cancer diagnosis* will be reviewed. An integrated system based on Bloch surface waves (BSW) sustained by 1D photonic crystals (1DPC) has been put forth. It combines the preparation of optimized dielectric thin film stacks on polymer substrates which exhibit significantly narrower resonance width than plasmon based sensors. Robustness of the BSW features with respect to the fabrication tolerances will be discussed. An optical system is designed and set-up to utilize the angularly resolved 'SPR-like' resonance tracking as label-free method in combination with the detection of surface wave coupled fluorescent emission to reveal the presence of fluorescent labels.This approach can be adapted to the case of angularly resolved resonance detection, thus giving rise to a combined label-free / labelled biosensor platform. It features a parallel analysis of multiple spots arranged as a one-dimensional array inside a microfluidic channel of a disposable chip, with fluids management achieved by means of a manifold integrating micro pumps and valves. Application of such a combined biosensing approach to the detection of cancer biomarkers in buffer solutions is reported.

Coffee break & Poster session 11:00 – 11:30

Morning Session 2– Chair: F.Michelotti 11.30 – 13:00

Biomarkers and Precision Medicine 11.30 – 12:00

Federico Bussolino
BILOBA Consortium, Università degli Studi di Torino and Institute for Cancer Research and Treatment
Candiolo, Torino, Italy

There are increasing interests in biomarker-driven precision medicine. Recently, dozens of molecular tests, including next generation sequencing and proteomics, have been developed to detect biomarkers that have the potential to predict response of cancers or other diseases to particular targeted therapies. However, detection of disease-related biomarkers and its implementation in clinical practice is far to be concluded. In my talk I'll review the successful strategies and the emerging problems for assessing functional consequences of molecular alterations and tools for finding applicable clinical trials, which exist to help bridge the gap between detection of disease-related biomarkerto the initiation ofbiomarker-matched targeted therapies.

Detecting Single Molecules with Optical Microcavities 12:00 – 12:30

Frank Vollmer
Max Planck Institute for the Science of Light
Erlangen, Germany

Dr. Frank Vollmer will present his results on advancing chip-scale biosensing capabilities with optical and plasmonic microdevices. He has developed a photonic-plasmonic biosensing platform that is capable of monitoring single DNA molecules and their interaction kinetics, hence achieving an unprecedented sensitivity for label-free detection with light. The platform can have important applications for studying the dynamics of molecular systems.

Round Table 12:30 – 13.00

What are clinicians and molecular biologists expecting from the technology providers?

Moderator: *Chiraz Frydman*
HORIBA Jobin Yvon Europe Research Centre
Palaiseau, France

The round table will challenge the attendees and the invited persons. We open the discussion with the audience by asking a challenging question to the attendees (role of the chairman).

Lunch &Poster Session **13:00 – 14:30**

Afternoon Session 1 – Chair: F.Michelotti **14:30 – 16:00**

Precision Medicine and Onco-Nano-Therapy 14:30 – 15:00

Patrizio Giacomini
National Cancer Institute
Roma, Italy

As known, tumors undergo genome-disrupting events such as mutations, rearrangements and amplifications. These are potentially 'actionable', e.g. they may be precisely targeted using small molecules and therapeutic antibodies, offering new, unprecedented therapeutic options, but requiring new 'personalized' approaches that differ in different diseases and patients. Precision approaches include: (a) companion assays to select subsets of eligible patients; (b) sensitive and non-invasive techniques to detect early-stage cancer and monitor relapse; (c) combination treatments to tackle molecular heterogeneity and resistance to therapy; (d) new low-toxicity drugs for long-term treatment; (e) new clinical response and follow-up criteria; and (f) cost-effective biotech platforms to implement the above. Manufacturing nano-objects on a supra-molecular scale may address these unmet medical needs. For instance, nanotechnology holds promise for ultra-sensitive and accurate detection of circulating nucleic acids (so-called 'liquid biopsy'), and for the construction of a new generation of nano-antibody-drug conjugates (nanoADCs) delivering large chemo-therapeutic payloads with fewer side effects. Thus, nanotechnology may ideally second the changing landscape of medical oncology.

Next-generation surface plasmon resonance for optical biosensing-based biomolecular interaction analysis and fragment based drug discovery: measurement principle and applications

15:00 – 15:30

Andrea Pigozzo
Alfatest
Milano, Italy

Surface-Plasmon-Resonance (SPR) is an optical phenomenon that provides a non-invasive, label-free means of observing binding interactions between an injected analyte and an immobilized biomolecule in real time. This technique is unmatched in its range of applications including: affinity and kinetics analysis, concentration assays, binding stoichiometry, thermodynamic analysis, study of interaction mechanisms and dependence of interaction on environmental conditions, antibody/fragment/small molecules screening, drug discovery, etc.
SensiQ Technologies provides Surface-Plasmon-Resonance (SPR) based platforms and disposable biosensors. SensiQ Pioneer introduces the innovative and unique Dynamic injection SPR (diSPR™) that provides enhanced information content in addition to binding affinity and kinetics characterization, without requiring the time-consuming individual concentration dilutions and injections necessary with other SPR instrumentations. SensiQ Pioneer is a cost-effective and highly sensitive platform for measuring binding affinity and kinetics for a broad range of biomolecules, including small molecules and fragments.

In Vivo selection of JARID histone demethylases inhibitors and their use to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation

15:30 – 16:00

Rodolfo Negri
SAPIENZA Università di Roma
Biology and Biotechnology Department "Charles Darwin"
Roma, Italy

Histone demethylases have a prominent role in epigenetic regulation and are emerging as potential therapeutic cancer targets. In order to discover inhibitors specific for H3K4 histone demethylation we set up a screening system which tests the effects of candidate small molecules inhibitors on a *S.cerevisiae* mutant strain which requires Jhd2 demethylase activity to efficiently grow in the presence of rapamycin. In order to validate the system we screened a library of 45 structurally different compounds designed as competitive inhibitors of α-ketoglutarate (α-KG) cofactor of the enzyme, and found that one of them, compound RS3195, inhibited Jhd2 activity in vitro and in vivo. The same compound effectively inhibits human JARID 1B and 1D in vitro and increases H3K4 tri-methylation in HeLa cells nuclear extracts. In order to improve inhibition efficiency and selectivity, we are now testing second generation molecules by surface plasmonic resonance (SPR) and activity assay.

Reference

C. Mannironi, M. Proietto, F. Bufalieri, E. Cundari, A. Alagia, S. Danovska, T. Rinaldi, V. Famiglini, A. Coluccia, G. La Regina, R. Silvestri, R. Negri, (2014) An High-Throughput In Vivo Screening System to Select H3K4-Specific Histone Demethylase Inhibitors. PLoS One. 9(1):e86002

Coffee break & Poster Session

16:00 – 16:30

Afternoon Session 2 – Chair: F.Michelotti 16:30 – 18:00

Surface plasmon resonance: some applications in pharmaceutical sciences 16:30 – 17:00

Paolo Bollella
SAPIENZA Università di Roma
Department of Chemistry and Drug Technologies
Roma, Italy

Surface plasmon resonance (SPR) is a label-free detection method which has emerged during the last two decades as a suitable and reliable platform in clinical analysis for biomolecular interactions. The technique makes it possible to measure interactions in real-time with high sensitivity and without the need of labels. This abstract reports some examples of works realized in our laboratory based on the use of SPR for drug analysis:

a) An SPRimmunosensor for the determination of 25-OH vitamin D obtaining a LOD of 2μg/ml. In order to enhance the sensitivity, the biosensor was modified with gold nanoparticles (AuNPs): the binding of 25OHD with AuNPs determines the amplification of SPR signal, allowing to lower the LOD down to 1 μg/ml, doubling the sensitivity. An alternative SPR method, based on the indirect determination of vitamin D by means of Vitamin D Binding Protein (VDBP), led to a further sensitivity increase reaching a LOD of 45 ng/ml which is really close to the fixed accomplishment for clinical diagnosis [1].

b) An SPR-based immunosensor for the real-time detection of cortisol and cortisone levels in urine and saliva samples. The method proposed here is simple, rapid, economic, sensitive, robust, and reproducible thanks also to the special features of the polycarboxylate-hydrogel-based coatings used for the antibody immobilization. Standard curves for the detection of cortisol and cortisone in saliva and urine are characterized by a detection limit less than 10 microg l^{-1}, sufficiently sensitive for both clinical and forensic use [2].

c) An SPR based immunosensor for the detection of insulin was obtained by means of a competitive immunoassay principle. In this case a bifunctional hydroxyl/thiol-functionalized fourth-generation polyamidoamine dendrimer-encapsulated Au nanoparticle (AuNP) was synthesized and immobilized on a mixed self-assembled monolayer-modified gold surfaceproviding an activated surface available to subsequently immobilize the antibody. The resulting Au NP dendrimer based immunosensorprovided an assay with a detection limit for analyzing insulin of 0.5 pM. This strategy allowed using this system to detect insulin in human serum [3].

d) The use SPR to study the interaction between liposomes with two anabolic androgenic steroids (AAS; i.e. testosterone, 19-nortestosterone) and the respective metabolism products. In this study, phytosphingosine, as ligand, was used to modify the surface to immobilize the liposomes on the surface.

References

[1] L. Carlucci et al., Biosensor and Bioelectronics 40 (2013), pp. 350-355.
[2] M. Frasconi et al.,Anal. Bioanal. Chem. 394 (2009), pp. 2151-2159.
[3] M. Frasconi et al.,Anal. Chem. 82 (2010), pp. 7335-7342.

Multiplexing high-throughput and label-free optical platform for biomolecular interaction monitoring

17:00 – 17:30

Franco Marabelli
Plasmore
Varese, Italy

We present a new bio-sensing technology based on the properties of a nanostructured plasmonic chip functionalized with ultrahigh-density peptide microarrays. The arraying technique is based on a Digital Micromirror Device (DMD)-driven photolithographic synthesis and allows the synthesis of up to 2 million individual and addressable peptides on a 2 cm^2. We interrogate these chips with several label-free detection technologies, such as imaging surface plasmon resonance imaging (iSPR) with an integrated optical device, surface enhanced Raman spectroscopy (SERS) and mass spectroscopy imaging (MALDI). Our objective is to allow real-time and simultaneously monitoring of protein interactions with hundreds of thousands of peptides at a time leading to rapid characterization and identification of peptide ligands to protein receptors, antibodies and other macromolecular peptide receptors.

This work was supported by European commission, Grant no. FP7-HEALTH-2011-HiPAD-278831

Round Table

17:30 – 18:00

What are pharmaceutical industries expecting from the technology providers?

Moderator: *Federico Bussolino*
Università degli Studi di Torino
Torino, Italy

The round table will challenge the attendees and the invited persons. We open the discussion with the audience by asking a challenging question to the attendees (role of the chairman).

POSTERS

P1 - Mid-Infrared Sensing with Epitaxial Semiconductor Antennas

M. Ortolani[1], M. P. Fischer[2], L. Baldassarre[3], A. Samarelli[4], K. Gallacher[4], J. Frigerio[5,6], V. Giliberti[1], E. Sakat[6], D. Brida[2], G. Isella[5,6], D. J. Paul[4], and P. Biagioni[6]

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P2 - Optical system for BSW based label-free and labelled analysis

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P3 - PIAD-deposition of multilayer coatings for Bloch surface wave optical biosensors

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P4 - Design of BSW stacks for combined label-free and labelled analysis

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P5 - Personalized care against sepsis with a novel optical biochip

B. Adinolfi[1], R. Bernini[2], F. Chiavaioli[1], A. Giannetti[1], I. A. Grimaldi[2], G. Persichetti[2], S. Tombelli[1], G. Testa[2], C. Trono[1], J. Eugen-Olsen[3,4], and F. Baldini[1]

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P6 - Label-free molecular recognition using Bloch surface waves on one dimensional photonic crystals

A. Sinibaldi[1], A. Anopchenko[1], N. Danz[2], P. Munzert[2], F. Sonntag[3], S. De Panfilis[4], A.Occhicone[1], and F. Michelotti[1]

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P7 - Compact SPR-Biosensor for the label-free detection of miRNA

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P8 - Label-free and fluorescence detection of antibodies on compact BLOCH-Biosensor

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P9 - Hydrodynamic induced cell rotation in microfluidic devices

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P10 - Pyroelectric effect investigation using microheater on $LiNbO_3$

S.Bhowmick[1], M.Iodice[1],M.Gioffrè[2], G.Breglio[2], M.Riccio[2], A.Irace[2], G. Romano[2], and G.Coppola[1]

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P11 - Optical nanotweezer for single nanoparticle trapping

D. Conteduca[1], F. Dell'Olio[1], T. F. Krauss[2], C. Ciminelli[1], and M. N. Armenise[1]

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P12 - Effect of random thickness variations on sensing properties of Bloch surface waves

A. Anopchenko[1], A. Occhicone[1], A. Sinibaldi[1], G. Figliozzi[1], N. Danz[2], P Munzert[2], and F. Michelotti[1]

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P13 – Comparison of the performance of the BILOBA platform to SPRi

E. Maillart[1]; F. Hibti[1]; S. Schmieder[2], N. Vollmer[2]; C. Frydman[1]

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P14 – Surface Functionalization of One Dimensional Photonic Crystals for Cancer Biomarker Detection

S. Rana[1], R. Chandrawati[1], A. Sinibaldi[2], N. Danz[3], P. Munzert[3], F. Michelotti[2], M. M. Stevens[1,4]

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P15 - Monitoring of Micro Fluidic Flow Conditions by Bloch Surface Waves

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ABSTRACTS
OF THE
POSTERS