DIGITAL MICROFLUIDICS AND NANOPARTICLE ENHANCED SURFACE PLASMON RESONANCE IMAGING (SPRI) FOR DNA AND RNA DETECTION

Giuseppe Spoto (Full professor)

gspoto@unict.it

Roberta D'Agata (Assistant Professor)

Laura Maria Zanoli (PhD Student)

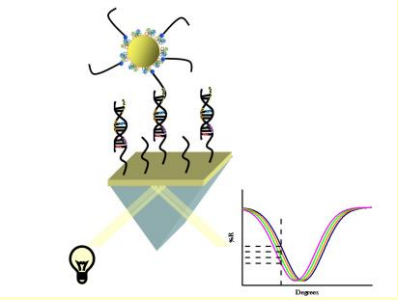

Fig.1 Detection of non amplified genomic DNA by advanced SPRI methods

The general objective of our research activity is to employ Surface Plasmon Resonance Imaging (SPRI) and digital microfluidic-based platform for DNA and RNA detection in various fields of life sciences for both applicative and research purposes.

Keywords: Genomic DNA, DNA sensing, Peptide nucleic acids (PNAs), PCR-free, Biosensors

1. Detection of DNA by PNA and Nanoparticle Enhanced Surface Plasmon Resonance Imaging

Innovative and ultrasensitive DNA detection approaches not requiring the PCR amplification of the genomic sample are crucial to advanced genetic diagnostic applications. Recently, we have shown that an ultrasensitive detection of non-amplified genomic DNA containing a target sequence as a minor component is obtained by using nanoparticle-enhanced Surface Plasmon Resonance Imaging detection and Peptide Nucleic Acids (PNA) probes. The experiments were carried out by using a PNA probe specifically designed to identify a selected genomic DNA sequence. Typically, no detectable variations in the SPRI signal are revealed at this stage by using target or control DNA concentrations lower than 1 μM. The detection of target or control DNA is obtained by using a sandwich hybridization strategy: a solution of AuNPs, conjugated to an oligonucleotide complementary to the final tract of the DNA target not involved in the hybridization with the PNA probe, is injected. The specificity of the target DNA adsorption is checked by comparing the nanoparticle-enhanced SPRI response with those obtained when the control sequences are allowed to interact with the surface-immobilized PNA. The DNA concentrations measured by the present method are in the range suitable for detecting DNA samples without amplification, which, in combination with microfluidics and miniaturized devices, could lead to the development of efficient tools for rapid, very specific and direct detection of DNA.

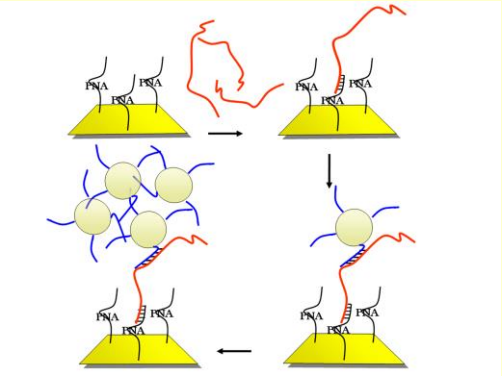

Fig.2 Pictorial description of the strategy used for the ultrasensitive nanoparticle-enhanced SPRI detection of the genetically modified DNA sequence

2. Digital Microfluidics-based Biosensor for DNA Detection

The our work shows the combined use of a droplet-based microfluidic system, PNA-MBs (peptide nucleic acid – molecular beacons) and fluorescence microscopy for the selective and sensitive detection of DNA using sample volumes in the range of 100-500 nL and fast, cheap and accurate sensing protocol. The experiments were carried out by using both oligonucleotides and PCR amplification products and were aimed at detecting DNA sequences of genetically modified soybean and different olive cultivar. The PNA improved selectivity in targeting complementary DNA sequences allowed an efficient detection and discrimination of single mismatched sequences by operating with sensitivities in the nM-µM range. The combined use of digital microfluidics, PNA beacon and fluorescence microscopy will be shown to provide an innovative and efficient platform for the simple and fast detection of 10^{-14}-10^{-15} moles of DNA samples.

Collaborations and Research Grants

- Neotron S.p.A (Modena); Prof. R. Marchelli, Prof. R. Corradini (University of Parma), Prof. R. Gambari (University of Ferrara), Prof. F. Biscarini (CNR-Bologna), Prof. A. Merkoçi (CIN2, Barcellona, Spain).
- A.T.S. s.r.l.Contract 2004.; Prin 2005, Neotron contract 2006, Meridionale Impianti S.p.A. 2006, Prin 2007.

Selected Publications

- D'AGATA, R., CORRADINI, R., FERRETTI, C., ZANOLI, L., GATTI, M., MARCHELLI, R., SPOTO. G. (2010) **Ultrasensitive detection of non-amplified genomic DNA by nanoparticle enhanced surface plasmon resonance imaging** BIOSENSORS & BIOELECTRONICS. Vol. 25, pp. 2095-2100. ISSN: 0956-5663.
- GRASSO; G., D'AGATA; R., ZANOLI; L., SPOTO G. (2009). **Microfluidic Networks for Surface Plasmon Resonance Imaging Real-Time Kinetics Experiments**. MICROCHEMICAL JOURNAL. Vol. 93. pp. 82-86. ISSN: 0026-265X.
- D'AGATA; R., CORRADINI; R., GRASSO; G., MARCHELLI; R., SPOTO G. (2008). **Ultrasensitive Detection of DNA by PNA and Nanoparticle-Enhanced Surface Plasmon Resonance Imaging**. CHEMBIOCHEM. Vol. 9. pp. 2067-2070. ISSN: 1439-4227.
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