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Photonic Crystal Surface Mode Imaging for Multiplexed Real-Time Detection of Antibodies, Oligonucleotides, and DNA Repair Proteins

Galina Nifontova¹, Evgeniia Gerasimovich², Fabrice Fleury³, Alyona Sukhanova^{1*}, and Igor Nabiev^{1*}

¹Laboratoire de Recherche en Nanosciences, LRN, Structure Fédérative de Recherche Cap Santé, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51100 Reims, France

²Laboratory of Nano-Bioengineering, Moscow Engineering Physics Institute, National Research Nuclear University MEPhI, 115522 Moscow, Russia

³DNA Repair Groupe, CNRS UMR 6286, US2B, Nantes Université, 44000 Nantes, France

Abstract. Sensors based on photonic crystal (PC) surface mode imaging are promising tools for label-free drug screening and discovery, diagnostics, and analysis of ligand–receptor interactions. Imaging of PC surface modes has been demonstrated to allow simultaneous real-time detection of multiple events at the sensor surface. Here, we report the engineering of a lateral-flow microfluidic assay where PC surface mode imaging is used for multiplexed detection of biomolecular targets (antibodies, oligonucleotides, and a DNA repair protein), as well as kinetic data on their interactions obtained without additional labelling or signal amplification. Our data demonstrate the suitability of the biosensing platform designed for ultrasensitive, quick, and low-cost detection and monitoring of interactions between different biomolecules.

1 Introduction

Label-free optical sensors based on photonic crystals (PCs) have been recently emerged as a promising tool for diagnostics, screening, and the analysis of ligand–receptor interactions. PCs represent nanometer-thick multi-layered stacks of dielectrics with periodic variation of their refraction indices in the optical wavelength range [1]. The binding events occurring at the PC surface lead to a shift of the periodicity and the wavelength of maximum reflectance, which is the operating principle of PC-based sensing. PCs are chemically and physically stable and can be completely regenerated after the use by plasma or UV treatment. The PC surface has been demonstrated to be easily modified with specific capture molecules using the silane and bioconjugation chemistry approaches [2] and to be more sensitive than the classical metal-coated surface plasmon resonance (SPR) biosensors [3]. Incorporation of a PC into a microfluidic chamber and imaging of the PC surface modes provide the possibility of real-time monitoring of multiple biomolecular interactions at the sensor surface [4].

Here, we present the results of designing of a lateral-flow microfluidic assay employing PC surface mode imaging for highly sensitive multiplexed detection of target biomarkers, such as oligonucleotides, antibodies, and other proteins, as well as kinetic data on their interaction obtained in the real-time mode without labelling. We also describe an approach to PC surface

functionalization with different protein/peptide capture molecules, the PC surface mode imaging bioanalytical procedure, and the proof-of-the-concept study on simultaneous detection of several proteins/peptides in a model panel.

2 Experimental Section

A one-dimensional PC consisting of alternating titanium dioxide (TiO_2 , $n_{\lambda 500 \text{ nm}} = 1.48$) and silicon dioxide (SiO_2 , $n_{\lambda 500 \text{ nm}} = 2.27$) layers ($\text{TiO}_2(\text{SiO}_2/\text{TiO}_2)_5\text{SiO}_2$) deposited onto the surface of a glass substrate ($n_{\lambda 500 \text{ nm}} = 1.52$) was prepared by electron-beam evaporation and plasma ion-assisted deposition as described earlier [5].

The PC surface was initially treated with UV and then aminosilanized and activated with a zero-length glutaraldehyde crosslinker. Protein/peptide capture molecules (antibodies, His-tagged biotin-binding proteins, and phosphorylated proteins/peptides) were spotted onto the pre-activated PC surface as a single-component protein/peptide array or in combination to form a model panel for exploring the possibility of multiplexed detection. After that, the PC was mounted in front of a colour camera to record surface mode changes during the analyte running, and the microfluidic chamber was assembled (Fig. 1). Analyte solutions were injected into the microfluidic chamber using a computer-controlled peristaltic pump. A parallel light beam ($\lambda = 500 \text{ nm}$) passed through the first polarizer ($+45^\circ$) and,

* Corresponding authors: alyona.sukhanova@univ-reims.fr; igor.nabiev@univ-reims.fr

after illuminating the mounted PC chip maintaining p-polarized PC surface modes at the indicated wavelength, through a prism in the Kretschmann-like configuration (Fig. 1). Reflected by the prism, the light came through the second polarizer (-45°) and was recorded by the colour camera. The optical signal resulting from the changes in the adsorption layer thickness and the position of the PC reflectance peak was then converted and normalized as a ratio of the blue and green pixel values. The regions of interest corresponding to the protein/peptide spots were selected in the obtained image, and multiple signals from the PC surface were simultaneously recorded and monitored over time.

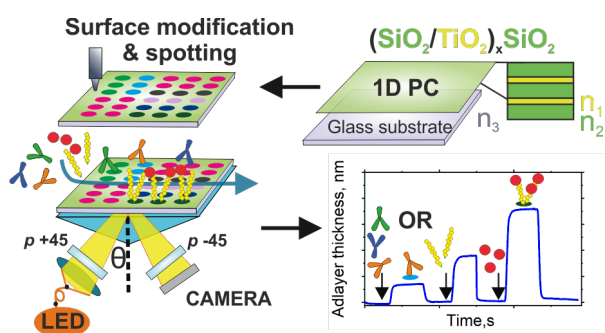


Fig. 1. The schematic diagram of a photonic crystal surface mode imaging biosensor and its operational principle. Abbreviations: LED, light-emitting diode; 1D PC, one-dimensional photonic crystal.

3 Results and Discussion

The sensing capacity of the PCs is strongly affected by the surface chemistry approach used for immobilization of capture molecules. Aminosilanization and treatment of the PC surface with the glutaraldehyde crosslinker allowed for covalent coupling and non-oriented immobilization of protein/peptide capture molecules. Conversely, initial attachment of biotin- or IgG-binding proteins to the PC surface ensured subsequent oriented binding of biotinylated capture molecules, such as oligonucleotides, and/or antibodies (IgG).

The bioanalytical procedure using the PC-based microfluidic sensing platform included initial introduction of the running buffer and blocking solution, subsequent injection of the analyte, and final dissociation of the assembled bioanalytical complexes (each step was performed until signal plateauing). The changes in the adlayer thickness over selected zones with time were recorded as sensorgrams demonstrating the kinetics of the observed interactions between immobilized capture proteins/peptides and analytes (antibody/oligonucleotide) molecules (Fig. 1).

The bioanalytical procedure developed here allows the detection of the capture molecules using both direct and sandwich assays. The sandwich assay detection was tested in an experiment on the binding of the DNA repair protein (RAD51) with its biotinylated substrates (oligonucleotides) preliminarily immobilized on the sensor surface via the biotin-binding protein (Fig.1).

The proof-of-the concept study on simultaneous detection of multiple biointeractions was performed by composing an array of 13 model proteins/peptides deposited in varying quantities on the PC surface. The optimal quantity of capture molecules ensuring the minimal area necessary for obtaining distinct sensorgrams was determined. The signal intensity detected over the regions of interest was shown to be distinguishable from the control zones containing inert proteins/peptides spotted there. The sensorgram recorded over control spots showed a drift over time by 0.013 SU. The detected biointeractions ensured a signal change of more than 0.02 SU. The spots for which a reliable PC surface mode image signal was determined provided the possibility of multiplexed detection of antibody analytes within the femtogram range without additional signal amplification.

4 Conclusions

Thus, we have demonstrated that PC-based sensors can be effectively used for simultaneous real-time label-free detection and monitoring of multiple protein-protein interactions. The engineered biosensing platform allows performing the analysis in the lateral flow microfluidic mode in the direct or sandwich format. The approach of PC surface modification developed here ensures oriented and non-oriented immobilization of the specific capture molecules and makes it possible to detect the analytes, in particular antibodies, within the femtogram range and obtain data on the kinetics of association/dissociation of the bioanalytical complexes. The designed sensor can be further modified for high-throughput screening providing quick ultrasensitive detection of larger panels of protein analytes.

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