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Galina Nifontova, Evgeniia Gerasimovich, Fabrice Fleury, Alyona Sukhanova, Igor Nabiev. Photonic Crystal Surface Mode Imaging for Multiplexed Real-Time Detection of Antibodies, Oligonucleotides, and DNA Repair Proteins. EPJ Web of Conferences, 2023, 287, pp.03007. 10.1051/epj-conf/202328703007. hal-04363661

HAL Id: hal-04363661 https://hal.science/hal-04363661

Submitted on 2 Feb 2024

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

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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 ligandreceptor 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 lateralflow 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 (TiO₂, $n_{\lambda 500 \text{ nm}} = 1.48$) and silicon dioxide (SiO₂, $n_{\lambda 500 \text{ nm}} = 2.27$) layers (TiO₂(SiO₂/TiO₂)₅SiO₂) deposited onto the surface of a glass substrate ($n_{\lambda 500 \text{ nm}} = 1.52$) was prepared by electron-beam evaporation and plasma ionassisted 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 (antibodies, His-tagged biotin-binding molecules proteins, and phosphorylated proteins/peptides) were spotted onto the pre-activated PC surface as a singlecomponent protein/peptide array or in combination to from 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 computercontrolled peristaltic pump. A parallel light beam (λ = 500 nm) passed through the first polarizer (+45°) and,

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after illuminating the mounted PC chip maintaining ppolarized 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 area necessary for obtaining distinct minimal 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.

This study was supported by the French National Research Agency through the FreeBioWave project, grant no. ANR-20-CE19-009-02). The part of the research related to the design and development of multilayered photonic structures was funded by the Russian Science Foundation, grant no. 21-79-30048.

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