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Multi-spot, label-free detection of biomarkers in complex media by reflectionless surfaces

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Abstract

The measurement of the intensity of light reflected by interfaces with extremely low reflectivity in water enables the label-free, multiplex quantification of the binding between immobilized probes (e.g. antibodies) and targets in solution using extremely simple instrumental set-up. Here we show that, despite its simplicity, the method enables label-free detection in complex samples characterized by high absorbance and turbidity. Diagnostic markers of Tomato spotted wilt virus are revealed in crude plant extracts of *Datura stramonium* leaves with early stage infections.

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Keywords: optical biosensor; label-free; immunoassay; reflective phantom interface; viral infection; tomato spotted wilt virus.

1. Introduction

Despite the continuous advances in bio-molecular detection and fluidic systems integration, the development of portable, high performance devices for rapid quantification of biomarkers in complex media still presents major difficulties, mostly because of the need to combine adequate sensitivity with low cost of production, operational

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simplicity and short time-to-result. In a previous work [1] we have introduced an extremely simple detection method, the Reflective Phantom Interface (RPI), which is based on the measurement of the light reflected by a functionalized surface having very low reflectivity. Various antibodies were immobilized in dozens of spots on the surface of an amorphous perfluoropolymer isorefractive with water by means of a multi-functional copolymer. The real-time, label-free quantification of molecular recognition processes was obtained from the analysis of the images of the light reflected by the sensing surface. The optical set-up is extremely simple, such that the flash LED and the CMOS camera of a smartphone can be employed to build a portable device based on the RPI method [2]. A limit of detection of a few pg/mm^2 was achieved targeting antigens widely used as markers for diagnoses of hepatitis B and HIV, corresponding to detectable concentrations in buffer solution or in serum as low as a few ng/ml.

Here we report the detection of a viral marker in a crude extract of leaves, a fluid sample characterized by high absorbance and turbidity. Tomato spotted wilt virus (TSWV) is an economically important pest able to cause severe crop losses in many crops worldwide, including many vegetables and ornamentals. TSWV is carried by insects and can infect different kinds of plants including the taxa of tomatoes and lettuce. In this study, the TSWV was inoculated to test plants of *Datura stramonium* and crude extracts obtained from the mechanical disruption of the leaves were analyzed and compared with extracts from uninfected plants. Despite the complexity and turbidity of the matrix, the RPI sensor provided enough sensitivity to detect in few minutes the virus from a single leaf with an early stage infection.

2. Materials and methods

2.1. Sample preparation

Plants of *Datura stramonium* were infected by inoculation 9 days before the tests. The infected material was ground with chilled inoculation buffer (Paul buffer: 150 mM phosphate buffer at pH 7.5, 5 mM Dieca, 1 mM EDTA, 5 mM sodium sulfite) and applied to the leaves of young plants with a small amount of carborundum. Then the plants were washed carefully in order to remove any residual abrasive powder. The test samples were obtained from leaves or a portion of leaves with apparent symptoms. The tissues were homogenized using an extraction bag (Bioreba) (Fig. 1A-B) and diluted 1:1 with Paul buffer.

2.2. Sensing surface

The RPI detection method enables the quantification of the amount of target molecules bound to probes immobilized on a surface providing extremely low reflectivity when in contact with an aqueous solution. In this study, the RPI substrate was made of a perfluorinated plastic with refractive index similar to that of water (Hyflon® AD, Solvay Specialty Polymer). The substrate was coated with a multifunctional copolymer of dimethylacrylamide (DMA) N-acryloyloxysuccinimide (NAS), and 3(trimethoxysilyl) propyl methacrylate (MAPS)—copoly(DMA-NAS-MAPS) [3] and spotted with different antibodies: two batches of polyclonal antibodies targeting TSWV (TSWV* and TSWV**) [4], a monoclonal antibody targeting hepatitis B antigen (HBS) and a polyclonal antibody targeting Pepino mosaic virus (PepMV). The antibodies were covalently immobilized in 200-μm spots by means of an automated noncontact dispensing system (sciFLEXARRAYER S5; Scienion AG).

2.3. Detection system

The theoretical and technical details of the RPI method are given elsewhere [1]. Briefly, the functionalized chip was placed into a cuvette containing a magnetic stir bar and the sensing surface was illuminated by the light of a LED at 455 nm. The image of the reflected light was acquired by a CCD camera (Fig. 1D). The acquisition started right after the addition of the sample into the empty cuvette, at room temperature. The brightness $u(t)$ of each spot as a function of time was converted into a parameter indicating the normalized surface density of target molecules $\Sigma(t) = (u(t)/u_0 - 1)^{1/2}$, where u_0 is the brightness of the bare chip surface.