Biosensors and Bioelectronics 58 (2014) 395–402

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A fast and simple label-free immunoassay based on a smartphone

Fabio Giavazzi [a,b,1], Matteo Salina [a,b,1], Erica Ceccarello [a,b], Andrea Ilacqua [a], Francesco Damin [c], Laura Sola [c], Marcella Chiari [c], Bice Chini [d], Roberto Cerbino [a], Tommaso Bellini [a,**], Marco Buscaglia [a,*]

[a] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, 20090 Segrate, Italy
[b] Proxentia S.r.l., 20135 Milano, Italy
[c] Istituto di Chimica del Riconoscimento Molecolare—C.N.R., 20131 Milano, Italy
[d] Istituto di Neuroscienze—C.N.R., 20129 Milano, Italy

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form
14 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Optical biosensor
Protein microarray
Immunoassay
Biomolecular detection
Reflective phantom interface
Point-of-care

ABSTRACT

Despite the continuous advancements in bio-molecular detection and fluidic systems integration, the realization of portable and high performance devices for diagnostic applications still presents major difficulties, mostly because of the need to combine adequate sensitivity with low cost of production and operational simplicity and speed. In this context, we propose a compact device composed of a smartphone and a custom-designed cradle, containing only a disposable sensing cartridge, a tiny magnetic stirrer and a few passive optical components. The detection principle is the previously proposed Reflective Phantom Interface that is based on measuring the intensity of light reflected by the surface of an amorphous fluoropolymer substrate, which has a refractive index very close to that of the aqueous sample solution and hosts various antibodies immobilized within spots. The reflectivity of dozens of spots is monitored in real time by the phone's camera using the embedded flash LED as the illumination source. We test the performance of the combined device targeting heterologous immunoglobulins and antigens commonly used as markers for diagnoses of hepatitis B and HIV. Target concentrations as low as a few ng/ml can be rapidly and robustly determined by comparing the rate of increase of the signal after the addition of the sample with that measured after the subsequent addition of a standard solution with known concentration. The features of the proposed system enable the realization of novel handheld biosensing devices suitable for those applications where multiple targets have to be rapidly detected even without the presence of trained personnel.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The continuous development of novel bio-detection approaches, potentially suitable for integration into compact, automated handheld devices (Myers and Lee, 2008; Holford et al., 2012), provides a vision of a forthcoming paradigm shift in the field of medical diagnostics (Gubala et al., (2012)) and, more generally, in all fields currently relying on lab-based biochemical analysis (Kozma et al., (2013)). In this context, the availability of high performance imaging sensors coupled with large computational power embedded into current smartphone devices represents an unprecedented opportunity for developing fully functioning, compact and low-cost instruments, avoiding expensive customized hardware fabrication. In recent years, this approach has been employed to realize a number of smartphone-based devices, including portable bright field, dark field and fluorescence microscopy systems (Tseng et al., (2010); Zhu et al., (2011b)), fluorescent cytometry platforms (Zhu et al., (2011a)), and automatic analyzers for quantifying the fluorescence (Lee et al., (2011)) or the scattering (You et al., (2013)) of labeling agents. However, few solutions have exploited smartphone functionality to realize label-free biosensors, which do not require labeling of the analytes, thus minimizing, in principle, sample processing and simplifying measurement procedures. In Gallegos et al. (2013) the phone camera was turned into a spectrophotometer for detecting the wavelength shifts in a photonic crystal biosensor, whereas in Huang and Ugaz (2013) the electrochemical dissolution of a chromium layer measured by imaging was shown to depend on the specific solution composition.

All of the above mentioned systems rely on some custom-designed, mobile phone attachment that takes advantage of the embedded camera and, when applicable, holds the sample in the

* Corresponding author. Tel.: +39 02503 30352; fax: +39 02503 30365.
** Corresponding author. Tel.: +39 02503 30353; fax: +39 02503 30365.
E-mail addresses: tommaso.bellini@unimi.it (T. Bellini), marco.buscaglia@unimi.it (M. Buscaglia).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.bios.2014.02.077
0956-5663/© 2014 Elsevier B.V. All rights reserved.

correct position. Generally, additional rather bulky external components are required, such as a proper illumination source or a pumping system, which complicate the development of truly portable designs. Moreover, the complexity of the required measurement procedures or of the particular sensing substrate employed prevents the realization of sensitive and simple assays based on low-cost disposable cartridges, suitable for large-scale diagnostic exams to be performed by untrained personnel.

Here we report the invention of a simple accessory, with less than 5 cm in depth, that transforms a smartphone into a portable biosensing device, enabling the parallel, label-free quantification of multiple markers in a few minutes and with minimum intervention of the operator. The detection method employed is the previously proposed Reflective Phantom Interface (RPI) (Giavazzi et al., 2013) that relies on the measurement of the intensity of light reflected by an interface with very low reflectivity in water ($\sim 10^{-5}$), therefore being almost invisible. The intrinsic simplicity of this approach is that it exploits the built-in LED and camera for the acquisition of the image of the light reflected by the sensing surface. The only component external to the phone that may require an electric supply, possibly a battery, is a tiny magnetic stirrer about 1 cm wide, which could be integrated into the main body of the device in a further engineering step. Using a cuvette-based, pre-assembled, disposable plastic cartridge, we demonstrate the performance of the system detecting fractions of nM of blood markers for HIV and Hepatitis B in serum.

2. Methods

2.1. The detection principle

In general, tiny amounts of material with a refractive index different from the surrounding medium can be easily observed by optical methods. The same principle applies if the surrounding medium is chemically heterogeneous, but has a homogenous index of refraction. Fluorinated polymer materials can be realized in order to have the same index of refraction as water, hence facilitating the optical detection of carbon-based compounds, which typically provide a substantially higher refractive index. In previous studies, we have exploited this concept with a twofold approach: (i) we used suspensions of nano-colloids made of fluorinated materials and estimated the amount of molecules adhering to their functionalized surfaces from the intensity of the scattered light (Ghetta et al., 2005; Prosperi et al., 2006; Morasso et al., 2010), and (ii) we proposed the RPI method (Giavazzi et al., 2013), which is based on the measurement of the intensity of the light reflected by a flat interface. Solution (i) is affected by possible limitations due to particle aggregation and very large area of the solid–liquid interface. Therefore, solution (ii) is highly preferable when dealing with multivalent interactions and very low analyte concentrations, typical properties of diagnostic markers. Accordingly, here we employ the RPI approach (ii) for the sensitive detection of antibodies and bio-markers. In this method, the interface hosts immobilized receptors while separating an aqueous solution from a solid, transparent material with a similar refractive index. In the appropriate conditions, the adhesion of molecules at this kind of interface causes an easily measurable increase in the intensity of reflected light, from which the amount of surface bound species can be estimated.

2.2. The device

The RPI approach has been previously demonstrated using a bench-top apparatus acquiring the light reflected by a perfluorinated plastic substrate iso-refractive with water (Hyflon® AD, an amorphous copolymer of tetrafluoroethylene, trademarked product of Solvay Specialty Polymers Italy) (Giavazzi et al., 2013). Basic components of the set-up were a collimated, thermally stabilized LED source (nominal wavelength of 625 nm, 14 nm FWHM bandwidth), a high resolution CCD camera, a magnetic stirrer and a system for the thermal stabilization of the sample. The optical set-up was designed to maximize the sensitivity of the RPI detection in a multiplex configuration: The LED light was expanded and collimated using a Köhler illumination scheme, providing an incident beam with high spatial uniformity and controlled divergence; and the collection optics allowed the formation of the image of the RPI surface on the CCD sensor with a suitable filtering, enabling the accurate selection of the light reflected in the specular direction. In this work we show that the same detection approach can be efficiently exploited using a minimal set-up based on an unmodified smartphone (Desire HD, HTC, Taiwan) combined with a simple custom-designed cradle hosting the measuring sample cell, a small magnetic stirrer and enabling the miniaturization of the optical layout required for the RPI detection. The device does not incorporate any temperature control system, and, in this configuration, the only external component necessary is the stirrer driver. The light emitted by the embedded, white light flash LED with a power of about 20 mW, is directed onto the bio-recognition surface and the reflected light is imaged by the backside illuminated CMOS sensor of the photo camera, having array size 3264×2448 and pixel size 1.4×1.4 μm. The optical and mechanical schemes of the combined device are shown in Fig. 1(a) and (b), respectively, and photographs are reported in Fig. 1(c) and (d). The optical design, described in Fig. 1(a), ensures the concomitant accurate selection of the reflected light and the high resolution imaging of the sensing surface. The LED light, partially collimated by the built-in converging lens is deviated, by means of a glass slab and a mirror, in order to impinge on the diagonal surface of a right angle prism made of Hyflon® AD, which is contained in the sample cuvette. The reflected light is then collected by an external close-up lens, spatially filtered and imaged on the camera sensor. The apertures along the optical path, including the small clear aperture of the smartphone camera lens, provide an effective spatial filtering of both the illumination and reflected beam, thus enabling an efficient rejection of the stray light. A polarizer placed on the reflection path also contributes to the reduction of background light. In fact, in this geometry the reflected light is strongly polarized because the angle of incidence is substantially coincident with the Brewster's angle.

The add-on assembly is composed of three parts made of black polyoxymethylene (Fig. 1(b)): The smartphone is inserted into a plastic holder with holes aligned with the LED and the camera detector positions; most of the optical elements are fitted in the sample cartridge holder attached to the smartphone holder; a mirror is encapsulated on the side of the cartridge holder by means of the third plastic part. In order to perform the measurement, the smartphone and the cartridge are inserted into the assembled cradle (Fig. 1(c)), the flash is turned on, and, exploiting the smartphone autofocus system, an optimized image of the sensing surface is obtained (Fig. 1(d)).

2.3. Surface preparation and execution of the measurement

Similarly to the previous study, the Hyflon® AD prism was coated with a multifunctional copolymer of dimethylacrylamide (DMA), N-acryloyloxysuccinimide (NAS), and 3-(trimethoxysilyl) propyl methacrylate (MAPS)–copoly(DMA-NAS-MAPS)—that both provides reactive groups for antibody immobilization and limits the unwanted nonspecific adsorption of serum components (Cretich et al., 2004). On the copolymer, different probe antibodies

Download English Version:

https://daneshyari.com/en/article/7233630

Download Persian Version:

https://daneshyari.com/article/7233630

Daneshyari.com