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Inexpensive optical system for microarray ELISA

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ABSTRACT

The use of antibody-based diagnostic testing has increased significantly over the past decade, giving rise to a wide range of diagnostic devices. At one end of the cost-range are rapid inexpensive point-of-care tests based on immunochromatographic strips which provide a qualitative positive or negative test outcome. On the other hand, quantitative tests generally require the use of dedicated and expensive laboratory instruments. There remains a need for diagnostic instruments and tests that can provide quantitative assessment of disease markers at low cost. This paper describes the development of a novel low cost optical device for reading colorimetric and fluorescent immunodiagnostic test results. This portable instrument uses a webcam to capture test results from a specially designed 16-well slide containing a miniaturized array of test spots. Arrays are illuminated with either LEDs or lasers, while transmitted or emitted light is captured through a long-pass filter, allowing two different types of optical measurement to be performed within the same device. This device was used to read results from an array of antibodies conjugated with either an enzymatic or fluorescent tag resulting in a colored or fluorescent readout.

1. Introduction

Immunoassays are used for the detection and quantification of antigens or antibodies in a biological sample and are the most widely used of all immunochemical techniques [1]. Immunoassays take advantage of the ability of antibodies to bind specifically to antigens where the degree of binding can be measured using an enzyme or dye-conjugated reagent. Assays performed using enzyme conjugates known as Enzyme-Linked Immunosorbent Assays (ELISA) are commonly used as a tool for clinical diagnostic measurements, drug screening, and for evaluating exposure to environmental agents [2]. ELISA tests are usually performed as discrete tests in which a single biomarker is measured. An alternative option is to develop multi-analyte immunoassays in which two or more biomarkers are measured simultaneously. The time required for a multianalyte immunoassay is generally the same as that required for a single biomarker, resulting in increased testing throughput [1,3,4].

Protein microarray technology provides a method to measure multiple biomarkers in a biological sample within a single experiment. In this technique, grids of microscopic target elements or spots are deposited onto a solid surface and exposed to a sample potentially containing the corresponding binding molecules. The degree of binding can then be assessed from the change in spot color (colorimetry) or fluorescent emission. Microarray immunoassays are of great interest in diagnostic applications due to their ability to analyze multiple biomarkers in parallel from individual samples thereby reducing the overall cost per test [1,5,6].

Most developing countries have an acute shortage of healthcare workers, and particularly of specialists with the necessary equipment for performing quantitative analyses of diagnostic tests. The few specialists that are available are concentrated in urban centers making them unreachable to the vast population in rural areas [7]. There is now a strong trend in clinical diagnostics towards decentralizing testing to various near-patient sites, with an urgent need for small, fast, inexpensive and easy-to-operate devices to enable more widespread monitoring of health and to reduce the costs and inefficiencies associated with healthcare testing [8].

The last decade has seen significant efforts into the development of novel immunoassay platforms using quantum dots [9], electrochemi-luminescent labels [10] and formats with complex microfluidics [11,12] which aim to minimize sample volume and maximize sensitivity. However, despite the huge number of platforms, none have emerged as a clear leader in the market.

These developments in immunodiagnostic platforms have largely been driven in response to the needs of the developed world [13]. The resulting diagnostic platforms are beyond the reach of poorly resourced laboratories in regions with the

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Table 1
Recent developments in immunodiagnostic platforms and estimated instrument costs.

Technology	Instrument	Average costs (USD)
Bead arrays	Flow cytometer	50,000
Chemiluminescent planar arrays	CCD-based reader	20,000
Fluorescent planar arrays	Laser scanner	75,000
Colorimetric planar arrays	High resolution scanner	5,000

majority of the world's disease afflicted people. Table 1 provides a summary of new platforms and cost-range for instruments required to use these technologies.

Relatively inexpensive optical devices such as LEDs, LASERS, and webcams have proliferated in recent years, making them appropriate for use in low-cost diagnostic devices. CMOS imaging devices such as those used in consumer webcams generally have very low manufacturing costs, and provide a high degree of flexibility, allowing the user to bring high-resolution image data directly into a computer application [14] or a portable device. These devices have also been reported as imaging systems for biochemical analysis [15–18].

In this paper, we report on the design and preliminary testing of an inexpensive webcam-based imaging device (WID) that is able to read immunodiagnostic ELISA tests results using two different detection methods: colorimetry and fluorescence. This device uses a consumer webcam housed in a light-tight box to measure the intensity of light being reflected or emitted either from a colored product or a fluorescent dye as a function of biomarker level in the sample, thereby allowing its concentration to be measured. The WID provides the sensitivity required to measure clinically relevant biomarker levels from biological samples. Our device uses an additional well into which a sample with known biomarker concentration is added as a reference to enable comparison with biomarker levels in the sample.

A device with the ability to quantify specific substances in biological samples using immunoassays utilizing low-cost, off-the-shelf components is a practical starting point for building a diagnostic system with applications in developing countries and rural healthcare centers that have minimum infrastructure.

2. Design and methods

2.1. Protein microarray setup

The protein microarray used to illustrate the efficacy of this diagnostic instrument is based upon the PictArray technology [19]. Arrays of 300 μm spots of mouse anti-human prostate-specific antigen (anti-PSA; Biocheck Inc., USA) were deposited onto a nylon-based membrane on a disposable plastic slide consisting of 16 individual wells (Fig. 1). Contact printing technology using quill pins was used to deposit the proteins on the slide surface [20]. Control spots of goat anti-mouse IgG–biotin and mouse anti-goat IgG and human IgG (Thermo, USA) were deposited to monitor reagent and test performance along with anti-PSA spots at concentrations ranging from 400 to 50 μg/ml diluted in a two-fold series (Fig. 2).

2.2. Imaging instrumentation

An imaging enclosure was constructed by laser-cutting 3 mm-thick acrylic plates that interlocked with each other, providing an easy assembly process (Fig. 3a). The enclosure was constructed from black acrylic in order to shield the system from outside light; acrylic pieces were assembled using methylene chloride solvent. An inexpensive consumer webcam (Creative VF0070, USA) was secured on top of the prototype above the slide at a distance of 22 mm allowing its field of view to capture the sample and reference well. Two mega bright white LEDs (OVL-5521, Multicomp) were positioned in parallel horizontally at a distance of 15 mm from the front of the slide and 25 mm apart from each other. The dispersion of light from these LEDs resulted in an even light field across the slide, allowing the webcam to capture reflected light for colorimetric detection (Fig. 3b). For fluorescent detection, two 30 mW 532 nm green beam lasers (Kangle Technology, China) were placed directly under each well as a light source to excite the dye molecules. The nylon based membrane in which the protein spots were deposited served as a diffusion filter, spreading the green laser beam across the area of the well containing the microarray. A long-pass red filter (cut-off λ_c=550 nm) (OG-550, Edmund Optics, USA) was used as the emission filter to block excitation light, while allowing fluorescent wavelengths (580 nm) to be recorded by the webcam (Fig. 3c). An optical-power/energy meter (Newport 1936-C) was used to measure the light power over the slide surface created by the LEDs (370 nW at λ=485 nm and 500 nW at λ=570 nm) and the green beam laser (40 mW at λ=532 nm).

In order to allow the user to manually select between the two detection methods and the area of the slide to be imaged, two handles were attached on each side of the device. One handle permitted the end-user to move the emission filter in front of the

Fig. 1. Isometric schematic of 16-well nylon-based plastic slide.

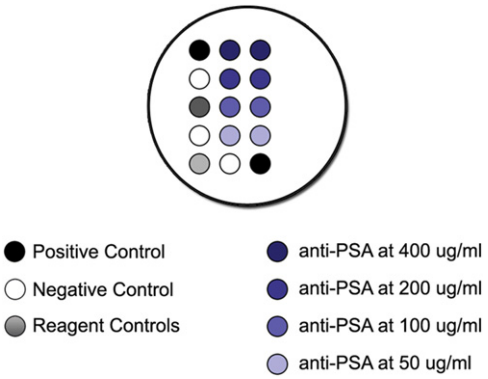

Fig. 2. Microarray layout.