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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

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

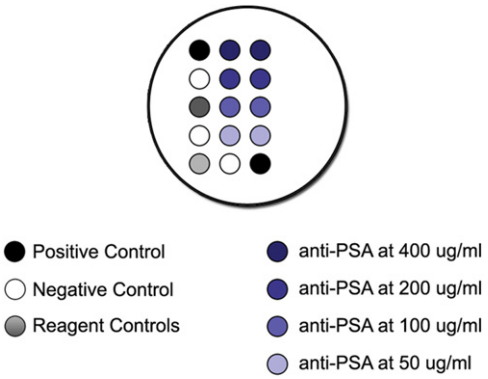

Fig. 2. Microarray layout.

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

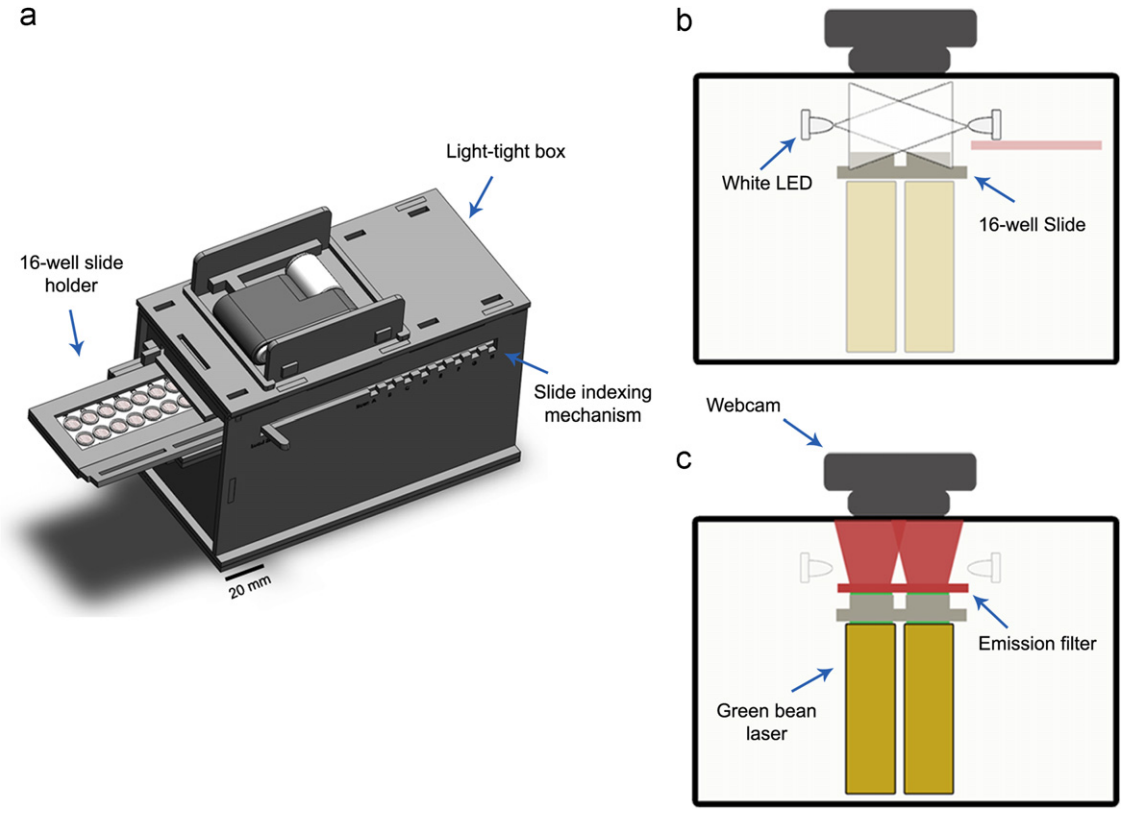

Fig. 3. Isometric drawing and arrangement of each component in the WID (a) External WID isometric drawing showing the manual slide indexing system along with the placement of the webcam. (b) Diagram of WID for colorimetry detection method. (c) Diagram of WID for fluorescence detection method.

webcam lens for fluorescent imaging, while the second handle served as a manual indexing system to guarantee reproducible incremental movement and positioning of the wells on the slide.

One convenient characteristic of this device is the ability to power the device entirely from a computer USB port by the incorporation of a 3.3 V voltage regulator (LM3940 National Semiconductor, US) to provide electrical power to the green laser diodes. This feature makes the WID a semi-portable device that can use a laptop to power the WID and acquire the images for analysis. A power switch and a detection method selection switch were included to allow manual setup of the device. Images for analysis were captured using National Instruments Visual Assistant version 8.6. The webcam was configured to capture 16-bit digital color images at a resolution of 1282×960 pixels and to save these as uncompressed bitmap image files (bmp) for later analysis.

2.3. Colorimetric assay method

After overnight storage at 4 °C, all wells in the 16-well slide with printed arrays were blocked with 1% casein (Vector Labs, USA) in 1X phosphate-buffered saline (PBS) containing 1% Tween-20 (PBS-T) for 30 min at 37 °C. All wells were rinsed three times with PBS-T. A two-fold dilution series of PSA standards (Biocheck Inc., USA) were added from a concentration of 100 ng/ml and incubated for 1 h at 37 °C. All wells were rinsed three times before addition of anti-PSA biotin and incubated for an additional hour at 37 °C, followed by incubation with Neutravidin–HRP (Thermo, USA). All wells were then rinsed three times with PBS-T. DAB substrate solution (Thermo, USA) was used to determine the amount of peroxidase activity at each spot. Residual substrate was washed off with PBS-T and the slide was dried and imaged using both the WID in colorimetric mode and a CanoScan 5600F scanner which was used as the reference standard for comparison.

2.4. Fluorescent assay method

The protocol for this method was as described in the colorimetric assay method, except Streptavidin–phycoerythrin (Moss Inc, USA) was used instead of Neutravidin–HRP and the slide was imaged by the WID in fluorescent mode and by a Fluorescent Scanner (GenePix 400B, Molecular Devices), used as a reference standard.

2.5. Data analysis

Data analysis consisted of three primary steps: (1) acquire images using adequate detection method settings, (2) extract RGB color mean values from the images corresponding to each spot, and (3) correct the extracted RGB values for overall background signal level. National Instruments Vision Assistant Software was used to define Regions of Interest (ROI) for each spot area from the captured images and to extract the RGB color mean values of all the pixels within the ROI. The mean RGB color values for each ROI were exported to Microsoft Excel. Duplicate test spot values were averaged and the overall background noise level was corrected by subtracting the average value of the print buffer spots. For colorimetric detection, the *Normalized colorimetric signal* was determined by calculating averages of the individual RGB signals and subtracting them from a full-scale value (255). For fluorescent detection, only the red and green mean values were used when images using the WID and GenePix 400B Fluorescent Scanner respectively since the remaining mean values were zero.

The data analysis algorithm used to determine whether the normalized signal intensity in the sample is clinically significant when compared to the one obtained from the reference is as follows:

$$C_{Test} = (I_{Test} \times C_{Ref})/I_{Ref} \quad (1)$$

$$f(C_{Test}) \begin{cases} \text{Negative} & C_{Test} < (C_{Ref} - CI) \\ \text{Ambiguous}(+/-), & (C_{Ref} - CI) < C_{Test} < (C_{Ref} - CI) \\ \text{Positive} & C_{Test} > (C_{Ref} - CI) \end{cases} \quad (2)$$

where,
C_{Test} = Sample concentration
I_{Test} = Sample intensity
C_{Ref} = Reference concentration
I_{Ref} = Reference intensity
CI = Confidence interval

3. Results

3.1. Response function and sensitivity

The response function of the WID was compared with that obtained using commercially available imaging devices. Fig. 4 shows the response function of the assay determined by the WID using both colorimetry (a) and fluorescent (b) readouts for PSA captured on anti-PSA antibody spots printed at 400 μg/ml.

In order to analyze the relationship between PSA concentrations and signal intensity in a background of human serum, a 3×3 microarray with duplicate spots of mouse anti-human PSA at 400 μg/ml including control protein spots (Fig. 5(a)), was deposited in each well of the 16-well slides. PSA-positive serum samples and normal samples were diluted 25-fold in blocker before being added to individual wells located on the left column of the slide. All wells located in the right column received 4 ng/ml PSA to serve as C_{Ref}.

Normalized mean test intensity values obtained from wells developed at 4 ng/ml PSA ($n=16$) were used to calculate the CI for each detection method. For a confidence coefficient of 95%, the CI was ± 0.3630 and ± 0.372 ng/ml for colorimetry and fluorescence, respectively. Subsequently, the data analysis algorithm described in Data Analysis was applied in order to determine if the tested samples had clinically significant levels of PSA (>4 ng/ml).

All normal samples and wells containing 2 ng/ml gave a "Negative" outcome. All but one of the eight positive samples and wells containing a concentration of 8 or 16 ng/ml of PSA were reported as "Positive". One positive sample gave an ambiguous result.

3.2. Comparison with ELISA

Concordance of results obtained using the WID with the colorimetric and fluorescent detection methods was established through comparison with results from a conventional ELISA Kit (Biocheck, USA) performed using the standard ELISA protocol suggested by the manufacturer. Data were analyzed and

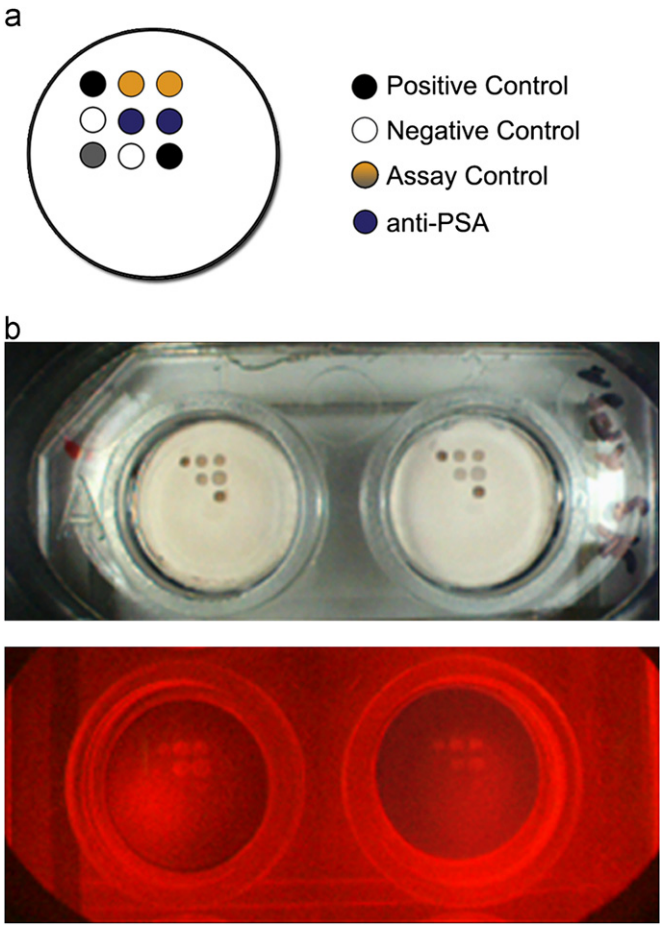

Fig. 5. (a) PSA microarray layout, (b) Positive Sample (Left Well) and Reference Well (Right Well) colorimetric and fluorescent assay image captured with WID.

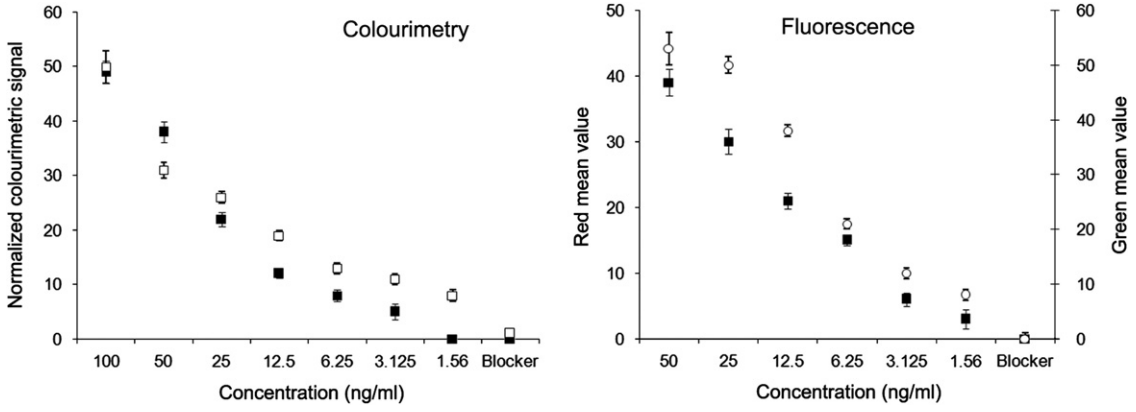

Fig. 4. Response function of WID prototype and commercially available imaging devices both for colorimetric and fluorescent detection methods for Mouse anti-human PSA printed at 400 μg/ml. (■) WID (□) CanoScan 5600F Scanner (○) Genepix 400B Fluorescent Scanner.

Table 2
Concordance results for positive and negative serum samples between WID and conventional ELISA kit.

	Positive	Ambiguous (+/−)	Negative
WID using colorimetry	7	1	4
WID using fluorescence	8	0	4
Conventional ELISA kit	8	0	4

compared giving a concordance of 91.6% and 100% for the colorimetry and fluorescent assays, respectively (Table 2).

3.3. Discussion and future work

Our objective was to demonstrate the feasibility of designing and developing a low-cost optical system able to read immunodiagnostic test results via multiple detection methods using off-the-shelf components. The PSA resolution limit of 4 ng/ml is within the clinically accepted range [21] making the WID suitable for detection of PSA.

Existing methods for accurate disease diagnosis are failing to meet the global healthcare needs, either because they are too expensive for widespread use or because they are not accessible in regions with the highest need. The combination of using immunodiagnostic tests and low-cost off-the-shelf components provides an integrated approach for laboratories in locations where resources are limited. The WID is ideal for this type of environment since it can be fabricated very easily, and the assembly processes does not require complex equipment or specialized training.

For most commercially-available diagnostic kits, a concordance percentage value greater than 90% is generally expected [22], making the WID a robust, reliable, and appropriate testing imaging device for immunodiagnostic test reading. Moreover, the use of an additional test cell as a reference with known biomarker concentration allows different panels for multiple diagnostic tests to be designed and analyzed providing a powerful tool for screening purposes without any modification of the current prototype. Furthermore, assay development time can be reduced by 1 h by using the fluorescent detection method since the enzymatic step of substrate conversion to a colored product is eliminated. Additionally, it gives the freedom to use a broader range of secondary antibodies tagged for fluorescent detection.

It is intended to automate the data analysis steps into a single routine to reduce the number of possible input-errors that can occur when defining the ROI for each spot. The input step for the routine could capture the image with the output being the normalized data. The biotin spot included in the array could be used as an alignment or anchor spot since it is meant to be visible regardless of the outcome of the sample being tested for an ease RGB color extraction.

The mechanism used to align the wells of the slide and switch between detection methods is implemented manually. A fully-automated device could be designed to speed up and reduce user-input for the imaging capture process of the slide. The integration of a microcontroller for device control and data analysis will only modestly increase the cost of the system.

Cell phones have become a household item with over 2.7 billion users across the globe and over 900 million of these phones contain a camera. These have demonstrated that they could become useful tools by transmitting images to off-site locations for diagnosis [23,24]. By incorporating a camera phone or a consumer electronic device (e.g. netbook, smartphone) capable of either analyzing the results within the device or sending them over existing communication channels could help to make this device a diagnostic tool more appropriate for use in remote locations that are difficult or impractical to access by trained physicians.

Moreover, a 50% beam splitter could be incorporated into the reader housing in order to reduce the number of laser diodes from 2 to 1 thereby reducing costs and power consumption. This design enhancement would allow the WID to be readily powered from portable power supplies such as rechargeable batteries making this diagnostic screening device more portable and energy-efficient.

In summary, this paper demonstrates the feasibility of building an affordable device that can be used to read the outcome of miniaturized ELISA-based tests. The use of this device, in conjunction with software to analyze the image and provide test results provides a cost-effective solution for measuring multiple markers of a single disease or multiple disease markers simultaneously, in a laboratory with minimum infrastructure costs. A wide variety of immunoassays can be designed and tested using this system making it applicable for a range of the most common infectious and chronic diseases in the world.

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References

[1] E.P. Diamandis, T.K. Christopoulos, Immunoassay, Academic Press, 1996.
[2] R. Biagini, C. Striley, J. Snawder, in: J. Van Emon (Ed.), Immunoassays and Other Bioanalytical Techniques, CRC Press, 2007.
[3] L. Kricka, The Immunoassay Handbook, in: D. Wild (Ed.), Nature, second ed., 2001, pp. 192–211.
[4] R.P. Ekins, J. Pharm. Biomed. Anal. 7 (1989) 155–168.
[5] M. Templi, D. Stoll, M. Schrenk, et al., DDT 7 (2002) 815–822.
[6] D.A. Hall, J. Ptacek, M. Snyder, Mech. Ageing Dev. 128 (2007) 161–167.
[7] H.S.F. Fraser, S.J.D. McGrath, BMJ 321 (2000) 465–466.
[8] M.K. Smith, Lancet 354 (1999) SIV26.
[9] K.E. Sapsford, D. Farrell, S. Sun, A. Rasooly, H. Mattoussi, I.L. Medintz, Sens. Actuators 139 (2009) 13–21.
[10] C.H. Yoon, J.H. Cho, H.I. Oh, M.J. Kim, C.W. Lee, J.W. Choi, S.H. Paek, Biosens. Bioelectron. 19 (2003) 289–296.
[11] J.H. Cho, S.M. Han, E.H. Paek, I.H. Cho, S.H. Paek, Anal. Chem. 78 (2006) 793–800.
[12] M. Zimmermann, E. Delamarche, M. Wolf, P. Hunziker, Biomed. Microdevices 7 (2005) 99–110.
[13] P. Yager, G.J. Domingo, J. Gerdes, Ann. Rev. Biomed. Eng. 10 (2008) 107–144.
[14] A. Dhir., The Digital Consumer Technology Handbook, Elsevier, 2004.
[15] N. Misawa, S. Takeuchi, IEEJ Trans. Electr. Electron. Eng. 6 (2001) 97–100.
[16] W. Wongwilai, S. Lapanantnoppakhun, S. Grudpan, K. Grudpan, Talanta 81 (2010) 1137–1141.
[17] J.P. Golden, F.S. Ligler, Biosens. Bioelectron. 9 (2002) 719–725.
[18] M. Solli, M. Andersson, R. Lenz, B. Kruse, Lect. Notes Comput. Sci. 3540 (2005) 380–391.
[19] S. Kumble, L. Choi, C. Lopez-Muedano, K.D. Kumble, J. Clin. Diagn. Res. 6 (2) (2012) 200–2006.
[20] M. Schena, Protein microarrays, Jones & Bartlett Learning, 2005.
[21] E.F. Petricoin, D.K. Ornstein, C.P. Paweletz, et al., J. Natl. Cancer Inst. 94 (2002) 1576–1578.
[22] Guidance for Industry, Bioanalytical Method Validation, US Food and Drug Administration, June 2012, 〈www.fda.gov〉.
[23] A.W. Martinez, S.T. Phillips, E. Carrilho, S.W. Thomas III, H. Sindi, G.M. Whitesides, Anal. Chem. 80 (2008) 3699–3707.
[24] S. Tachakra, X.H. Wang, R.S. Istepanian, Y.H. Song, Telemed J.E. Health 9 (2003) 247–257.