PARALLELIZATION OF CHIP-BASED FLUORESCENCE IMMUNO-ASSAYS WITH QUANTUM-DOT LABELLED BEADS

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ABSTRACT

This paper presents a novel optical concept for the read-out of a parallel, bead-based fluorescence immunoassay conducted on a lab-on-a-disk platform. The reusable part of the modular setup comprises a detection unit featuring a single LED as light source, two emission-filters, and a color CCD-camera as standard components together with a spinning drive as actuation unit. The miniaturized lab-on-a-disk is devised as a disposable. In the read-out process of the parallel assay, beads are first identified by the color of incorporated quantum dots (QDs). Next, the reaction-specific fluorescence signal is quantified with FluoSpheres-labeled detection antibodies. To enable a fast and automated read-out, suitable algorithms have been implemented in this work. Based on this concept, we successfully demonstrated a Hepatitis-A assay on our disk-based lab-on-a-chip.

Keywords: FIA, parallel assays, beads, quantum dots, centrifugal microfluidics

INTRODUCTION

The miniaturization of devices for clinical diagnostics towards point-of-care applications is subject of numerous approaches. Typically, such "lab-on-a-chip" or "micro total analysis" systems (μTAS) feature a full process integration, a high reproducibility, reduced consumption of sample and reagents as well as short times-to-result, and ease of handling [1]–[6]. Besides the development of the analytical chips which are preferably disposable, detection devices have been developed which basically comprise an actuation and a read-out unit [7][8].

To further advance towards a comprehensive picture of the patient's health state, especially in the field of emergency diagnostics, the parallel determination of a set of relevant biochemical markers in a given sample would drastically accelerate the benefit of these μTAS.

As a special type of "lab-on-a-chip" systems, a "lab-on-a-disk" [9]–[12] exploits centrifugal forces generated by a spinning drive to pump liquids. On its way radially outwards, the sample is processed [13][14] and finally reaches a detection chamber. While the spinning drive, as the actuation unit, is a robust and reusable "macro" component, the "micro"-parts are here reduced to their functional essence, i.e. passive microstructures which can be fabricated in a very economic fashion, e.g. by industrial scale micromachining techniques such as hot-embossing or injection molding.

The implicit advantages of centrifugal microfluidics like its robustness and reproducibility together with its cost efficiency recommend this platform for point-of-care applications, especially in the field of emergency as well as clinical diagnostics [15]–[17].

In our approach, a set of fluorescence immunoassays (FIAs) is run on a lab-on-a-disk with the use of color-tagged beads. Prior to loading them into the disk, the beads are functionalized with the relevant capture antibodies. In the automated read-out of the assay results using an image processing software, each bead is localized and spectrally identified in a first "tag-color" CCD image. A second fluorescence picture acquires compound fluorescence intensity of each bead.

QUANTUM-DOTS AS OPTICAL BEAD-TAG

Quantum-dot (QD) crystallites excel with their broad-band excitation and their narrow-band luminescence. Their spectrum can be tuned by their diameter ($d_{QD} \approx 5$ nm) during fabrication [18]–[20]. We use the large number of optically distinguishable QDs made of CdSe for bead identification in parallel assays (Fig. 1). To this end, the QDs are first suspended with polystyrene (PS) beads ($d_{bead} = 150$ μm) in an organic solvent. The solvent swells the PS, thus incorporating roughly 10^5 quantum-dots in the polymer matrix of the bead. By vacuum drying, the pores shrink to irreversibly capture the QDs.

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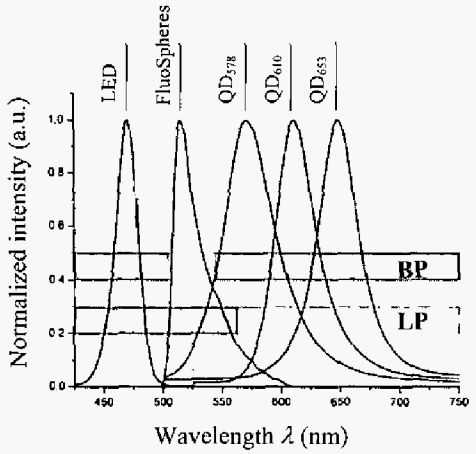

Fig. 1 Quantum-dots as optical tag of the beads: Three types of quantum-dots (QD_{578}, QD_{610}, QD_{653}) are incorporated in beads and their luminescence spectra are recorded while being commonly excited by an LED ($\lambda_{peak} = 475$ nm). To optically distinguish the bead-tag from the signal of the high-intensity FluoSpheres™ [24] as assay label two optical filters are required. A long-pass filter (LP 570) selects the luminescence of the QDs to record the bead-tag while a band-pass filter (BP 505–540) exclusively transmits the signal of the fluorochrome as a measure of the assay result.

PARALLEL FLUORESCENCE IMMUNOASSAY

As an off-disk preparative step of the parallel bead-based FIA, each type of QD-encoded bead is first functionalized with designated capture proteins (Fig. 2). Next, the QD-beads representing different capture antibodies are mixed, suspended in a buffer solution, and loaded into the inlet reservoir of the disk (material: Cyclic Olefin Copolymer, COC). The disk also comprises a detection chamber and an outlet drain (Fig. 3 – A).

The liquid is then transported by the centrifugal force F_v into the detection chamber. Here, the outlet drain of the detection chamber establishes a geometrical barrier to the beads as the depth of the chamber d_{out} is smaller than the bead diameter (i.e., $d_{out} = 90\ \mu m = 0.6 \cdot d_{bead}$) (Fig. 3 – B). On the other hand, the depth of the detection chamber $d_{det} = 180\ \mu m$ is chosen only slightly larger than d_{bead} to aggregate a statistically distributed monolayer in the detection chamber. This configuration allows a direct optical access to all aggregated beads for a CCD camera mounted in a top view position of [21][22].

The subsequent on-disk fluorescence immunoassay (FIA) is implemented by a custom-made spinning protocol $\nu(t)$ controlling the flow rates and the incubation times at high accuracy. After the delivery of the sample and washing, the FluoSpheres™ labelled, detection antibodies [24] specifically react with the previously formed antibody-antigen complex.

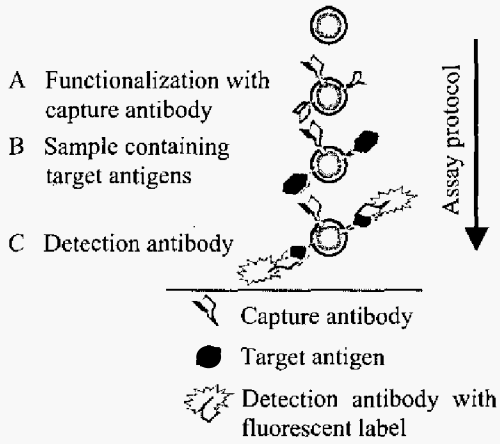

Fig. 2 Concept of a fluorescence immunoassay (FIA) based on beads as solid-phase. (A) The beads are first functionalized with a certain capture protein and then loaded into the detection chamber on-disk. (B) Next, the sample containing the target antigens is transported into the detection chamber and the specific antigen-antibody complex is formed. (C) After washing, the added detection antibodies which are labelled with fluorochromes specifically bind to these antigen-antibody complexes.

The read-out procedure as the final step of the parallel FIA significantly deviates from the procedure of the conventional single-channel assay (Fig. 3 – C). The fluorescence intensity has to be spatially resolved to allow the assignment to the type of bead in the subsequent step. We first acquire an image of the bead-tag distribution (LP 570, Fig. 1) by illumination with a high-power LED ($\lambda_{peak} = 475$ nm) [23] and capture the image with a color CCD-camera [25]. With the same light source, the distribution of the assay-specific fluorescence intensity is recorded in a second image with a bandpass filter (BP 505–540, Fig. 1).

READ-OUT PROCESSING ALGORITHM

The post-processing of the first image locates the centers and identifies the color-IDs of the QD-labelled beads using a Hough transform (Fig. 4) [26].

The recognition of beads starts by taking the Euclidean distance between the vector of a pixel and reference vectors of a QD-type in RGB-space. This distance is compared to a threshold R_i and, if accepted, the current pixel is transformed to the Hough space of the bead centers for a given bead radius. Here, each pixel-value within the bead-radius of the current pixel is increased by one. The bead centers are then determined by finding the maxima in Hough space. Additional robustness is achieved by constraining the distance of neighbouring beads to more than the bead radius. The programmed routine lasts less than 15 s and displays a reliability better than 80 % .

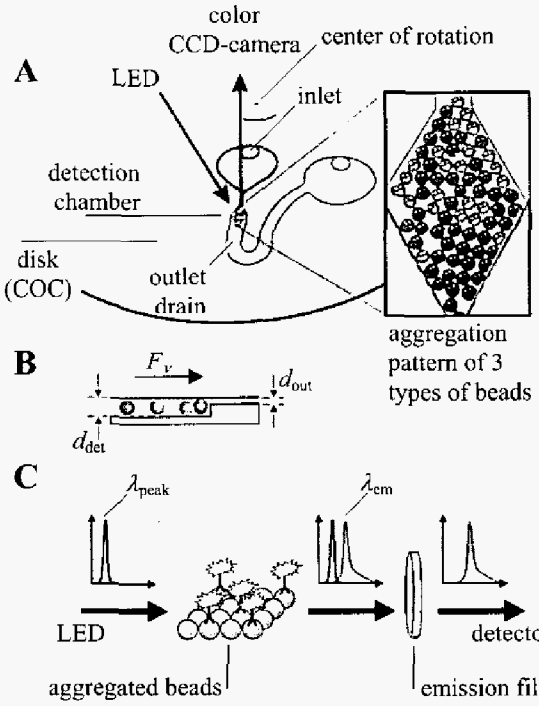

Fig. 3 Read-out of a bead-based fluorescence immunoassay (FIA) on-disk. (A) Beads as solid phase of the FIA are loaded into the detection chamber of the disk where they aggregate as a monolayer (B) in front of a geometrical barrier. (C) After the assay is conducted, the beads are excited by the light of an LED [23] with peak wavelength λ_{peak}. The induced light (λ_{em}) of the fluorochrome [24] is spectrally filtered by the emission filter to suppress the reflected light of the exciting LED and then directed towards a color CCD-camera [25] as detector. Note that the photographs originally features distinguishable colors.

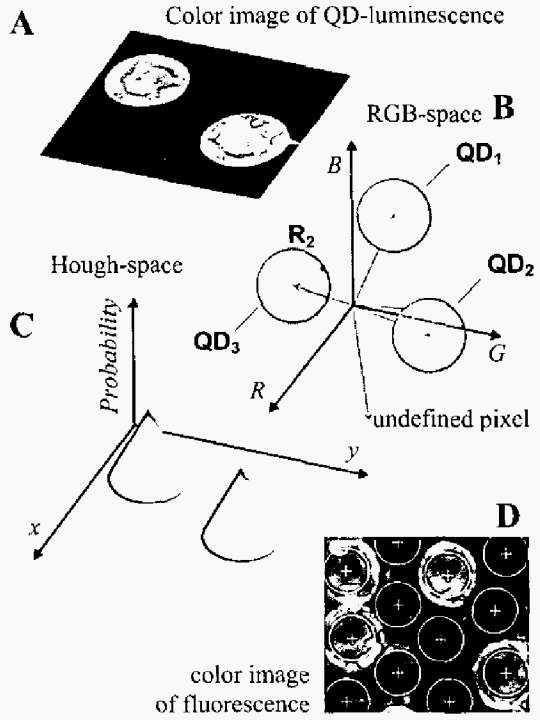

Fig. 4 Concept of the image processing routine for a three-fold assay: (A) each pixel of the QD-luminescence image is transferred as a vector into RGB-space and the Euclidean distance d to the reference vectors $\mathbf{QD_i}$ (i = 1, 2, 3) of the QD-colors is measured. (B) For $d < R_i$, with R_i as a color threshold, this pixel is assigned to QD_i and transformed to the Hough space [26] of the bead centers for a given bead radius. (C) The bead centers are then determined by finding the maxima in Hough space. (D) From the fluorescence image, the assay results are measured by averaging the fluorescence signal within a concentric circle to suppress signal overlapping.

RESULTS

To demonstrate the feasibility of a parallel, disk-based FIA with the QD-encoded beads, a dilution series is run. It starts from standard human serum (Paul Ehrlich Institute) containing a known (calibrated) concentration of Hepatitis-A antibodies (Fig. 5). The measured curve clearly allows to distinguish the state of long-term protection by Hepatitis-A vaccination (10 international units per ml according to the WHO regulations).

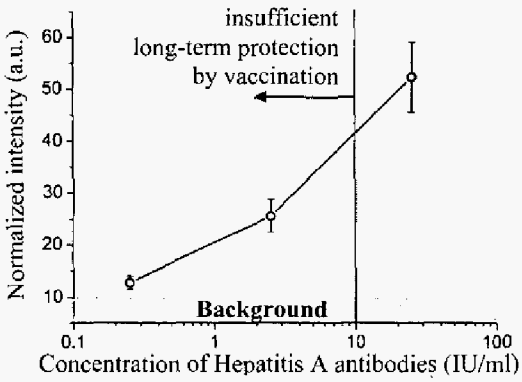

Fig. 5 Calibration curve of the Hepatitis A assay: the result clearly allows to determine the state of protection by Hepatitis-A vaccination according to the WHO regulation (limit at 10 international units (IU) per ml).

CONCLUSION

We realized a rugged optical setup assembled by conventional components which is suitable for parallelized fluorescence immuno-assays. Color encoding is implemented by incorporating quantum-dots into the beads while high-intensity FluoSpheres™ are coupled to the detection antibodies. We successfully demonstrated a Hepatitis-A assay on a miniaturized lab-on-a-disk platform. In contrast to complex high-end setups such as sequentially operating flow cytometers, our setup well complies with the technical and economic demands of diagnostic point-of-care applications.

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REFERENCES

[1] Sia, S.; Linder, V.; Parviz, B.; Siegel, A.; Whitesides, G. *Angew. Chem. Int.* **2004**,43, 498–502.

[2] Vilkner, T.; Janasek, D.; Manz, A. *Anal. Chem.* **2004**, 76, 3373–3386.

[3] Ducrée, J.; Zengerle, R. *FlowMap – Microfluidics roadmap for the life sciences*, **2004**, Books on Demand GmbH: Norderstedt, Germany, ISBN 3-8334-0744-1, www.microfluidics-roadmap.com.

[4] Reyes, D.; Iossifidis, D.; Auroux, P.; Manz, A. *Anal. Chem.*, **2002**, 74, 2623–2636.

[5] Auroux, P.; Reyes, D.; Iossifidis, D.; Manz, A. *Anal. Chem.*, **2002**, 74, 2637–2652.

[6] Examples in point-of-care diagnostics: Careside Analyzer®, Careside Inc, USA; Biosite® Biosite Inc., France; Pelikan SunTM of Pelikan Technologies Inc., USA; Chempaq Analyzer, Chempaq A/S, Denmark; i-STAT Portable Clinical Analyzer, i-STAT Corp., USA.

[7] Triage Meter Plus, Biosite Inc., USA, www.biosite.com.

[8] Cardiac Reader, F. Hoffmann-La Roche Ltd., Switzerland, www.roche.ch.

[9] Schembri, C.; Burd, T.; Kopf-Sill, A.; Shea, L.; and Braynin, B., *Journal of Automatic Chemistry*, **1995**, 17 (3), 99–104.

[10] Puckett, L.; Dikici, E.; Lai, S.; and Madou, M. *Anal. Chem.*, **2004**, 76, 7263–7268.

[11] Ekstrand, G.; Holmquist, C.; Örleforn, A. E.; Hellmann, B.; Larsson A.; and Andersson, P. *Proc. μTAS 2000 conference*, **2000**, 311–314.

[12] Ducrée, J.; Brenner, T.; Grumann, M.; Bessler, W.; Stelzle, M.; Messner, S.; Nann, T.; Rühe, J.; Moser, I.; Zengerle, R. *Bio-Disk - A Centrifugal Platform for Integrated Point-of-care Diagnostics on Whole Blood*, **2003**, www.bio-disk.com.

[13] Brenner, T.; Häberle, S.; Zengerle, R.; and Ducrée, J. *Proc. μTAS 2004 conference*, **2004**, 566–568.

[14] Grumann, M.; Geipel, A.; Riegger, L.; Zengerle, R.; and Ducrée, J. *Lab on a Chip*, **2005**, accepted for publication.

[15] LabCD™, Tecan Group Ltd., Switzerland, www.tecan.com.

[16] Gyrolab Bioaffy CD, Gyros AB, Sweden (formerly Pharmacia Inc., USA), www.gyros.com.

[17] Piccolo, Abaxis Inc., USA, www.abaxis.com.

[18] Nann, T.; Riegler, J. *Chem. Eur. J.*, **2002**, 8(20), 4791–4795.

[19] Kucur, E.; Riegler, J.; Urban, G.; and Nann, T. *J. Chem. Phys,*. **2003**, 119(4), 2333–2337.

[20] Nann, T.; Mulvaney, P. *Angew. Chem. Int. Ed.*, **2004**, 43, 5393–5396.

[21] Grumann, M.; Schipppers, P.; Dobmeier, M,; Häberle, S.; Geipel, A.; Brenner, T.; Kuhn, C.; Fritsche, M.; Zengerle, R.; and Ducrée, J. *Proc. IMECE (ASME)*, **2003**, 2003-41427.

[22] Grumann, M.; Dobmeier, M.; Schippers, P.; Brenner, T.; Zengerle, R.; and Ducrée, J. *Lab on a chip*, **2004**, 4, 209–213.

[23] Luxeon III Star, Lumileds Inc., USA, www.lumileds.com.

[24] F8774 (λ_{exc} = 505 nm, λ_{em} = 515 nm), Molecular Probes, USA, www.probes.com.

[25] Axiocam MRc, Carl Zeiss AG, Germany, www.zeiss.de.

[26] Young, T. Y. *Handbook of pattern recognition and image processing*, **1986**, Academic Press.