

HIGHLY-INTEGRATED, LOW-COST IN-VITRO DIAGNOSTIC PLATFORM FOR MINIATURIZED ASSAY DEVELOPMENT

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ABSTRACT

The paper describes a low-cost microfluidic platform with integrated micropumps and optical biosensor for the development of miniaturized assays for in-vitro diagnostic and point-of-care applications. The platform consists of disposable microfluidic cartridges incorporating all parts necessary for carrying out immunoassays (including reagents) as well as a readout instrument providing both the optical, fluorescence based readout and the electrical interface for pump control. The feasibility of the system for running immunoassays is demonstrated by a CRP and PSA based assay carried out completely inside such a cartridge.

KEYWORDS: micropump, immunoassay, in-vitro diagnostics, disposable, system integration, TIRF biosensor

INTRODUCTION

For point-of-care (PoC) applications, transferring biological assays from the tube to an integrated, miniaturized system is of increasing importance. Bio-companies and –start-ups are rapidly developing assays and kits. The paper presents a technological platform for scaling down such assays to a miniaturized, modular microfluidic device which allows for the integration of electrochemical and optical biosensors, and can be used for completely different bioassays.

MICROFLUIDIC CARTRIDGE WITH INTEGRATED PUMPS

The microfluidic cartridges provide integrated low-cost micropumps which have been significantly improved to previous publications [2]. The pumping functionality is facilitated by the electrochemical generation of gas. The generated gas deflects a membrane into the fluidic reservoirs, driving the liquid into the channel system towards the sensing region.

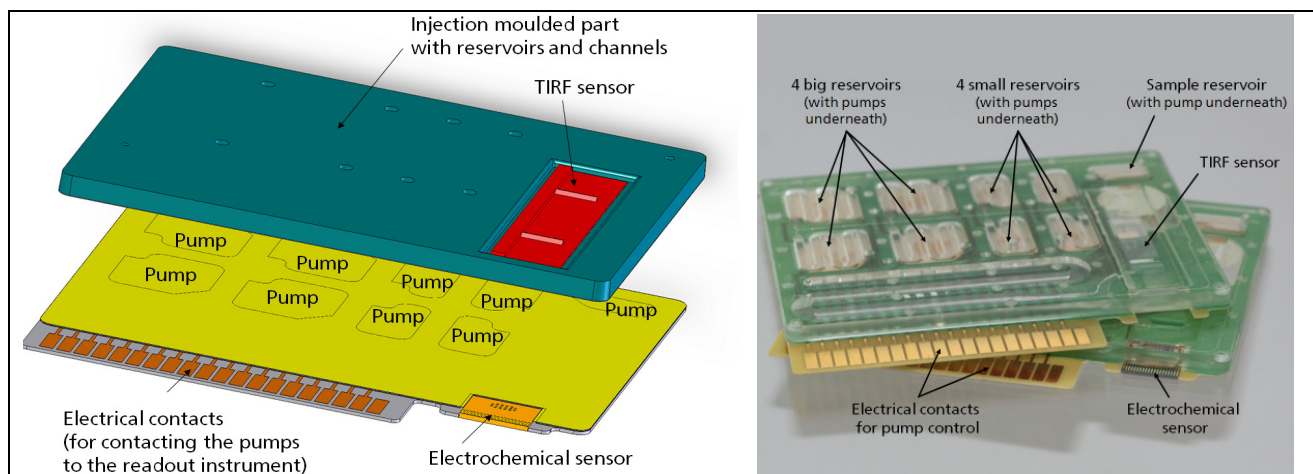


Fig. 1: Microfluidic cartridge with integrated reservoirs, low-cost micropumps and optical biosensor (left: schematic representation, right: photographs of the fabricated cartridge; version with electrochemical and optical biosensor).

To illustrate the working principle of the cartridges, Fig. 2 shows an emptying sequence of the first three reservoirs. For better visibility, all reservoirs have been filled with inked water. A detailed description is given in [3].



Fig. 2: Pumping process: Emptying of the first three reservoirs by the integrated micropumps. The progress of pumping can clearly be seen by the different colors in the sensor region on the left side. Please note that there is no additional interface to the cartridge than electrical contacts.

Only technologies suitable for mass fabrication have been involved in the design of the cartridges, allowing for an easy upscaling to real Point-of-Care applications without the otherwise “typical” system change from “lab-scale-development” to “mass fabrication”. The whole process chain for the fabrication of those cartridges is currently under development.

OPTICAL BIOSENSOR AND READ-OUT INSTRUMENT

While the cartridges are designed to work with different kinds of biosensors (both optical and electrochemical, see Fig. 1), for the example application of this paper an injection-moulded fluorescence based optical biosensor will be used (Fig. 3).

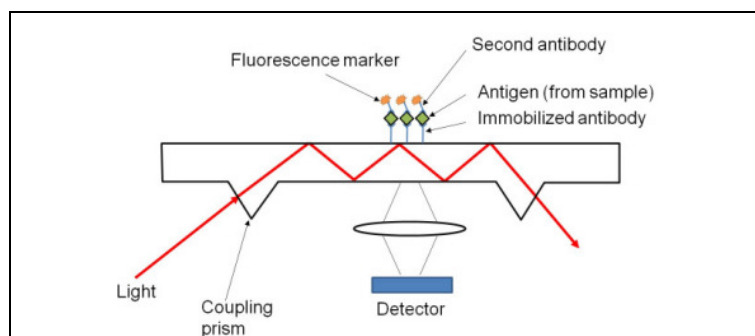


Fig. 3: Working principle of the total internal reflection fluorescence (TIRF) sensor. The sensor has been fabricated by injection moulding.

The sensing principle of the optical biosensor is based on total internal reflection fluorescence (TIRF) which has described elsewhere [1]. In short, light is coupled into a thin polymer foil via a coupling prism and propagates inside the foil by total internal reflection. The evanescent field of the light excites fluorochromes on top of the foil. A fluorescence image is recorded by a CCD detector underneath the spotted region of the TIRF sensor. Due to the short penetration length of the evanescent field, the background signal from the bulk liquid can significantly be reduced, allowing for higher signal-to-noise ratios.

A read-out and control device (Fig. 4) has furthermore been developed for optical readout and control of the integrated micropumps. The whole assay can thus be programmed by software and does not need any fluidic interfaces to the cartridge.

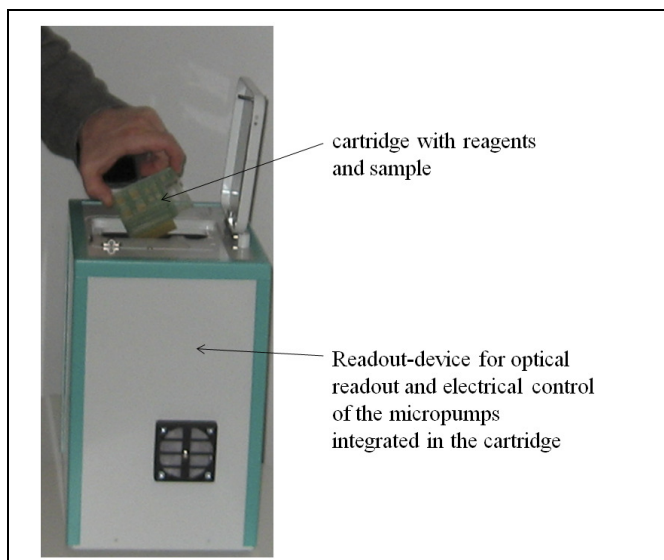


Fig. 4: Instrument for optical readout and electrical control of the integrated micropumps.

EXPERIMENTAL

To proof the feasibility of the platform, a complete ELISA (enzyme linked immuno sorbent assay) has been carried out in the cartridge (Fig. 5) for two different markers: the inflammation marker CRP (C-reactive protein) and the cancer marker PSA (prostate specific antigen). The spotting layout on the top surface of the TIRF sensor is shown in Fig. 6a). Besides antibodies for the markers, also antibodies for two different hormones as well as positive and negative controls have been spotted. The spotted TIRF sensor was placed in the already fully assembled cartridge.

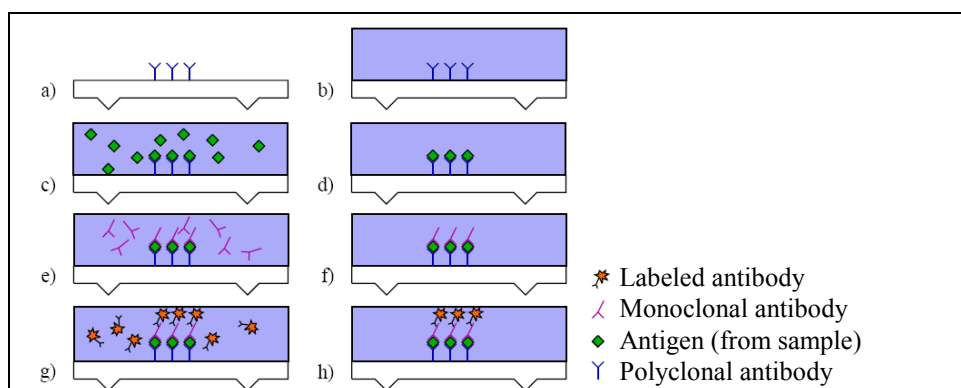


Fig. 5: Steps of the immunoassay carried out in the cartridge. Steps b), d), f), and h) are washing steps with buffer.

The integrated liquid reservoirs of the cartridges were filled with buffer solutions and antibody solutions (see also Fig. 5). At this stage, the cartridge can be stored for a certain time before usage. Just before running an assay, the sample reservoir of a cartridge was filled with antigen solution and sealed. Concentrations of 10µg/ml CRP antigen and 1µg/ml PSA antigen were used, both in PBS (phosphate buffered saline) buffer.

Each cartridge was then inserted into the readout device (Fig. 4). The pumping sequence for carrying out the assay shown in Fig. 5 had been programmed before.

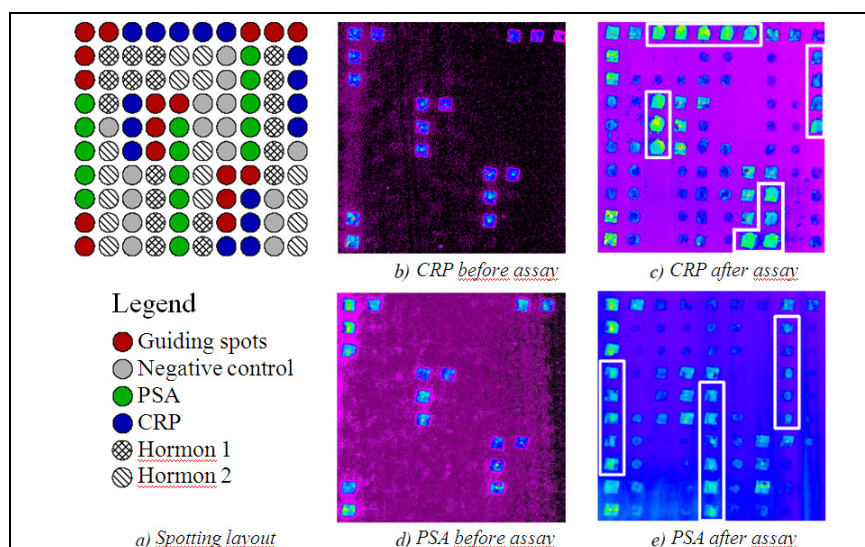


Fig. 6. Spotting layout and false-color fluorescence images before and after assays. Both assays have been run by the integrated micropumps completely inside a cartridge; readout and pump-control were carried out using the readout instrument shown in Fig. 4. The shape of the spots is caused by a pin-tool with a square-like cross section.

Fig. 6 b) and d) show the fluorescence images obtained by the readout device before the CRP and PSA assay respectively. Fig. 6c) and e) show the fluorescence images after carrying out all steps of the assay. It can clearly be seen that the fluorescence intensities of the according spots increased for the different assays.

CONCLUSION

A fully integrated, polymer based cartridge has been presented which is able to run ELISAs without any fluidic interfaces to the outside world. Thus, also no external liquid reservoirs or pumps are necessary. The cartridges have been used with a polymer fluorescence sensor for carrying out two different immuno assays.

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