Two Microfluidic Platforms for Miniaturization, Integration and Automation of Assays

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Abstract

Two different platform concepts for microfluidic miniaturization, integration and automation of biochemical assays are presented. First, unit operations for **batch-wise** nucleic acid analysis on the **centrifugal microfluidic platform** are demonstrated, including unit operations for DNA extraction, aliquoting and real-time PCR. Second, the newly developed continuous **phase transfer magnetophoresis platform** is introduced. It enables **continuous** online process monitoring, demonstrated by implementation of unit operations for DNA extraction.

1.0 Introduction

The prospect of integrating, miniaturizing and automating laboratory analytical and diagnostic protocols is expected to result in a substantial annual market growth for products based on microfluidics of 18 % in the next years [1]. At current, worldwide, thousands of researchers contribute to the growing field of microfluidic concepts and applications. Yet, only a few standards are defined in terms of interconnections, production processes *etc*. In our opinion, for exploring the huge potential of different applications in the field of lab-on-a-chip, a component based microfluidic approach is much too slow and the R&D effort much too expensive. In addition, the best performance one can expect from such a *component oriented solution* will be far behind the possibilities of any *integrated system approach* or in other words a *microfluidic platform approach*. Such microfluidic platforms should offer an adequate number of *microfluidic unit operations* that can be easily combined to build application specific microfluidic systems. In addition, those systems should be producible using cost efficient standard technology. Several platform concepts are already under development today, as described in [2]. In this paper, we focus on two different examples of microfluidic platforms that can be both applied for automation of nucleic acid analysis protocols. One of the platforms is operated in a batch mode, the other in a continuous mode.

2.0 The Centrifugal Microfluidic Platform

Centrifugal microfluidics has the charm of relying only on one rotor and a structured polymer disk for liquid control [3]. Since no connections to pumps are required, this platform promises robust, easy to use and contamination free integration of analytical protocols. There are numerous unit operations for the centrifugal microfluidic platform, including structures for valving, metering, mixing, switching and aliquoting of liquids as well as protocols for binding, washing, sedimentation and separation of bio-molecules and cells.

We present the set of unit operations for our centrifugal microfluidic platform [4] which is required for the implementation of nucleic acid based testing. These unit operations provide solutions for solid-phase extraction, aliquoting, and real-time PCR. A DNA extraction structure with a yield of 290 ng ± 80 ng DNA per 100 µL of eluate from a 32 µL lysed blood sample (Fig. 1) was realized. The extracted DNA showed no real-time PCR inhibition compared to a reference extraction (QIAamp DNA Blood Mini Kit) [5].

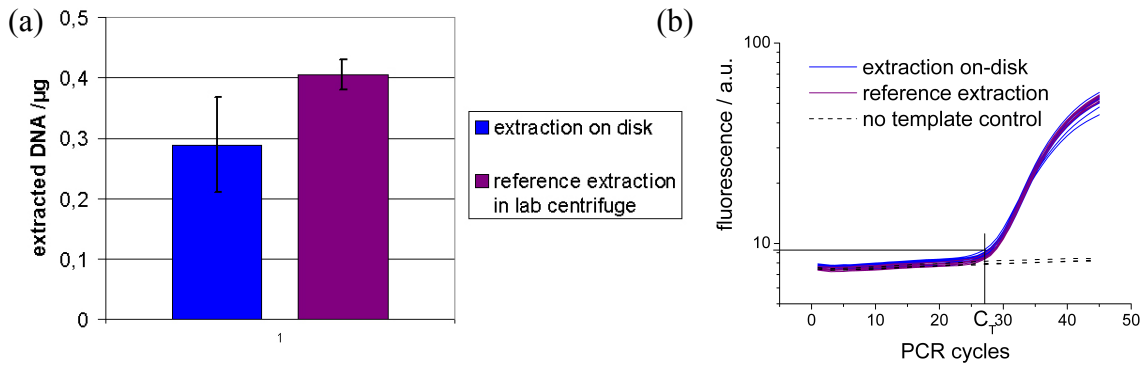

Figure 1: Performance of DNA extraction on a centrifugal microfluidic disk versus a standard reference extraction with spin columns in a lab centrifuge. (a) DNA quantity: extracted DNA mass. (b) DNA quality: Amplification plot of ~ 600 starting copies of DNA. No inhibition was observed at the DNA extracted on-disk compared to the reference [5].

For aliquoting the extracted DNA a novel aliquoting structure was designed and tested. A 105 µL volume was split into 16 aliquots with a volume CV of 3.0 % [6]. The aliquoting structure does not require local surface modifications and works for a large range of liquids (including ethanol, liquids containing detergents and aqueous solutions). Real-time PCR compatibility was demonstrated by a foil cartridge in a commercially available centrifugal thermocycler [7] (Fig. 2).

Since mobile diagnostic systems need to be extremely energy efficient, future Lab-on-a-Chip solutions will favour isothermal protocols rather than thermocyling based approaches. Thus, we demonstrate the full integration of the isothermal recombinase polymerase amplification (RPA) in a centrifugal microfluidic system and its use for the detection of bacterial pathogens such as methicillin resistant *Staphylococcus aureus* [8].

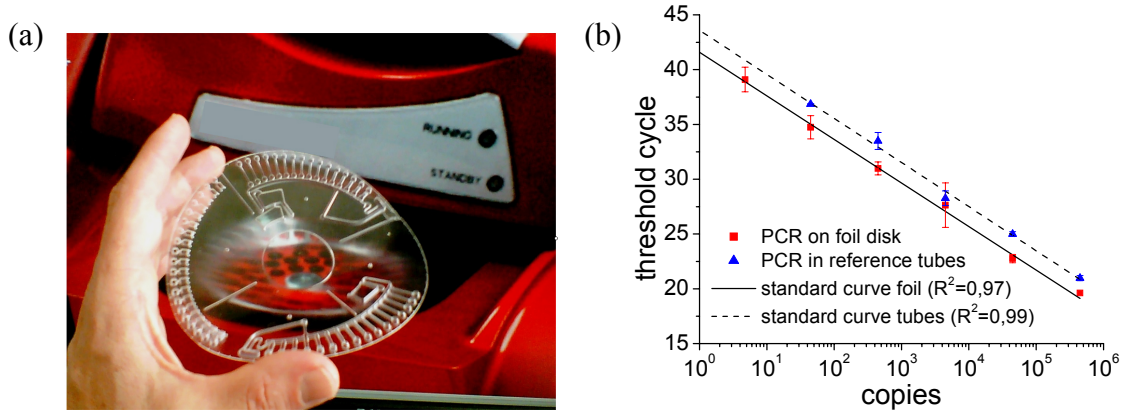

Figure 2: Real-time PCR on a microfluidic disk. (a) Blow moulded foil disk with aliquoting structure, and thermocycler. (b) Standard curve of a real-time PCR dilution series of the *Exfoliative A* gene performed on disk and, as a reference, in standard PCR tubes.

3.0 The Phase Transfer Magnetophoresis Platform

For continuous monitoring of biological samples, e.g. for on-line process control in fermenters, a continuously operated microfluidic platform is required. For this purpose, we developed the phase transfer magnetophoresis (PTM) platform. Superparamagnetic capture beads are transferred by the magnetic force of a rotating permanent magnet between liquid-liquid-interfaces of laminar flowing reagents, allowing continuous processing of a sample. As application example, a DNA extraction protocol has been realized on the platform [9].

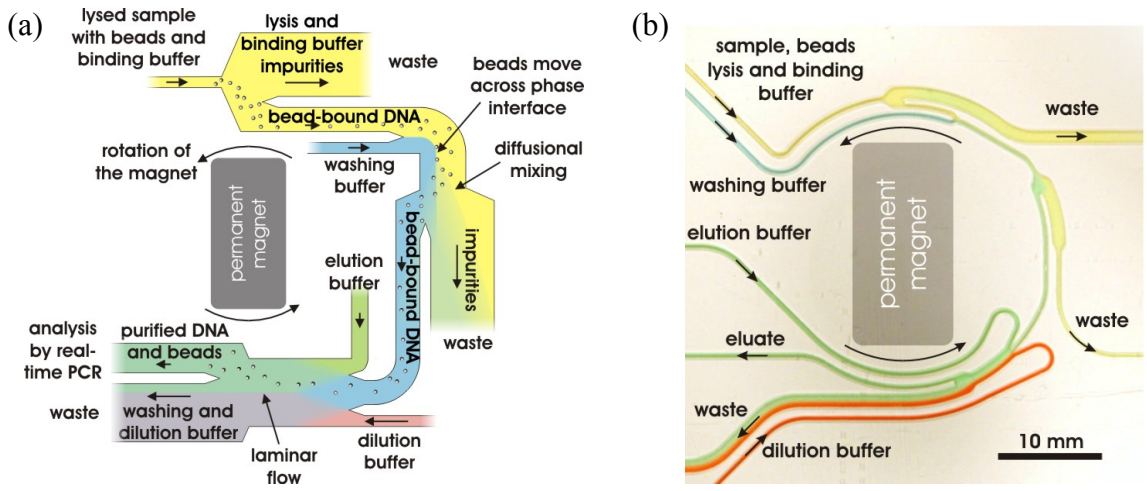

Figure 3: The phase transfer magnetophoresis Platform. (a) Schematic view of the microfluidic chip for continuous DNA extraction. (b) Realization of the platform in a polymer chip. Liquids are coloured for better visibility [9].

The working principle is depicted in Fig. 3. The first step of DNA extraction and purification after lysis of the cells is binding of the DNA onto the superparamagnetic beads in order to separate the DNA from impurities such as enzymes and/or cell debris. After the separation a washing step is included to remove the high salt buffers required for DNA binding as well as any residual impurities. As a last step the beads are transferred into the elution buffer where the DNA dissociates from the beads to allow real-time analysis. First results showed that DNA extracted with the PTM platform could be amplified by real-time PCR, although with a slight inhibition of 2 cycles at a starting concentration of $6.5 * 10^5$ cp/µl (3.15 ng/µl) [9].

4.0 Conclusions

The two presented platform concepts target batch - and continuous nucleic acid analysis. The platform approach ensures that the presented unit operations can be integrated into a fluidic network and are compatible with standard production processes. This approach enables ongoing improvements on the presented platforms while allowing the integration of established structures. This greatly reduces the development time for the realization of new protocols, since only small parts of the integrated assay have to be developed from scratch. Once a sufficient base of platform unit operations can be realized with a standard production process, a powerful and universal tool is generated that allows the integration, miniaturization and automation of a large number of biochemical assays.

5.0 Acknowledgements

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