

MICROFLUIDIC APPS ON STANDARD LAB-INSTRUMENTS

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ABSTRACT

Here we promote a new way of thinking: Designing microfluidic chips that can be processed on standard instruments which are already present in typical laboratory setups. Those instruments could be standard lab centrifuges, real-time PCR cyclers or even second generation sequencers. We call this approach the Microfluidic App approach [1].

KEYWORDS: Lab-on-a-Chip, Centrifugal microfluidics, Sample Preparation, Genotyping, Next-generation sequencing

INTRODUCTION

The perspective to automate and miniaturize assays or whole laboratories by using microfluidics or lab-on-a-chip devices led to a large research boom in microfluidics over the last two decades. However, only a limited number of commercially available devices surfaced from this boom so far. In our view, one aspect that impedes market uptake is the proprietary, complex, and expensive instrumentation that is often required to operate microfluidic chips. This approach is costly and also contributes much to the development risk.

Here we promote a new approach: Designing microfluidic chips that can be processed on standard instruments. Those instruments could be

- standard lab centrifuges,
- real-time PCR cyclers, or even
- second generation sequencers.

Similar to software Apps for smartphones those “Microfluidic Apps” could potentially reduce the costs for lab automation to the price of disposable plastic test carriers [2].

EXAMPLES

As a first example we report on our new LabTube platform for automation of biological assays. The LabTube can be designed for several tests, such as ELISA, DNA and protein extraction and is operated on standard lab-centrifuges. It is based on three stacked disposable microfluidic cylinders as depicted in Fig.1. Pen mechanics, actuated by centrifugal forces, rotate cylinder II. This allows for opening and closing of fluidic paths through the stack and thus liquid routing.

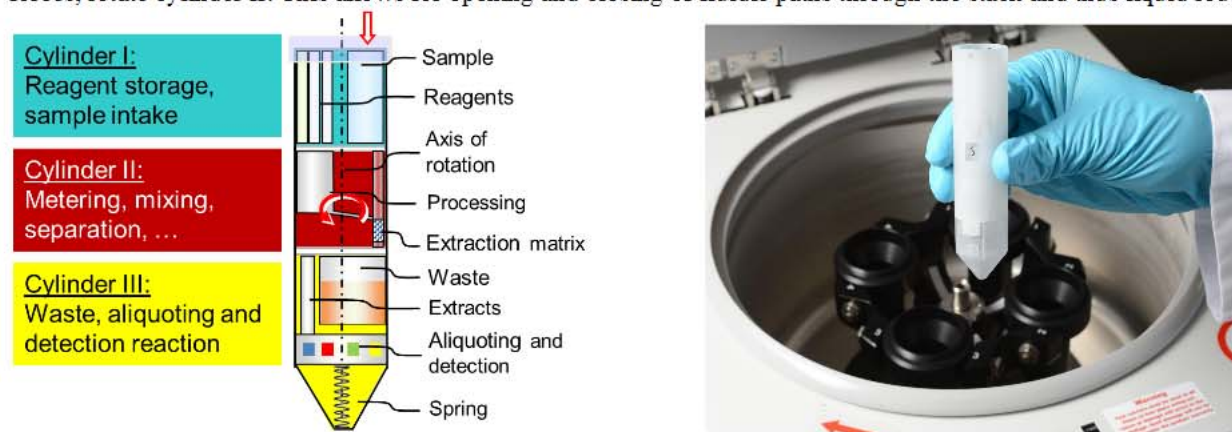


Figure 1: Concept and first demonstrator of the LabTube for the swinging bucket rotor of a standard lab centrifuge (Photo by Bernd Mueller).

Cylinder I contains pre-stored liquid reagents and an interface for sample intake. Cylinder II contains lancing structures for sequential release of liquids from Cylinder I and can be equipped to perform unit operations such as

metering, mixing, separation, and column based extraction of target molecules. Cylinder III separates the waste liquids from the target liquid and provides space for the integration of analytic reactions.

As a first demonstration, a fully automated column-based DNA extraction from human blood was performed. Cylinder I was loaded with lysis buffer, binding buffer, washing buffer and elution buffer (DNA Blood Mini Kit, QIAGEN, Germany) and a human blood sample was added. A silica column for DNA binding was integrated in cylinder II. After fully automated processing in the centrifuge, DNA eluates were taken from cylinder III and analyzed for the human growth hormone (HGH) gene by real-time PCR (Fig. 2). In comparison to a manual reference sample, 27 - 45 % DNA was recovered with the LabTube. Analysis of the different unit operations showed that mixing in the LabTube is crucial for DNA extraction and is the main reason for the lower DNA yield of the LabTube. Therefore, future work will concentrate on optimization of the mixing unit operation for DNA extraction in particular.

Major advantages of the LabTube platform are the possibilities for on-site reagent pre-storage and release, combined with the flexibility for automated processing of different assays with varying volumes, e.g. ranging from 50 µl to 1 ml of sample volume for DNA extraction and the “plug and play” operation in a standard laboratory centrifuge.

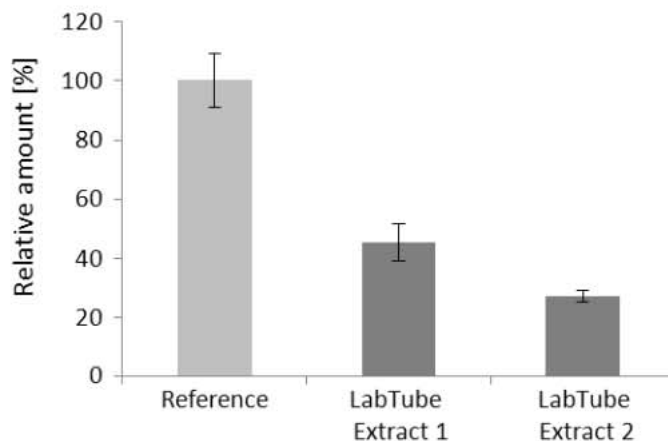


Figure 2: Results of extracted DNA quantified by real-time PCR on HGH gene. DNA from human blood samples was extracted manually with standard protocol or fully automated using the LabTube. The DNA extraction yield is referenced to the manual control. Error bars correspond to three technical replicates of the PCR.

As a second example of microfluidic Apps, we present a microfluidic cartridge for automated genotyping that runs on a standard real-time PCR cycler [4] (see Figure 3). This microfluidic App automates aliquoting of the sample and mixing with pre-stored primers and probes and is processed and fluorescently read out by the standard instrument.

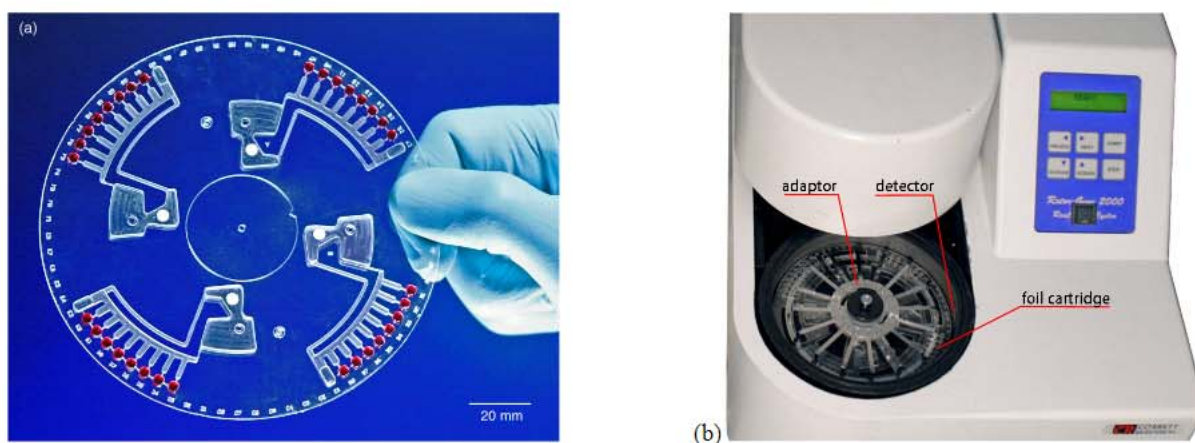


Figure 3: Design of the LabDisk foil cartridge. (a) Photograph of the LabDisk (reaction wells are highlighted with red ink, through holes for air flow are not shown); (b) Assembly of the LabDisk cartridge in the modified centrifugal thermocycler Rotor-Gene 2000 (open top cover position) (figures taken from [4]).

As a third example we present a new approach to fabricate whole genome microarrays based on using next generation sequencing technology [5]. The approach is based on performing highly parallel PCR reactions in a picowell array

(PWA) and simultaneously immobilizing the generated PCR products in a covalent and spatially-resolved manner onto a microscope slide via solid-phase PCR (SP-PCR) (see illustration in figure 4). Even single DNA molecules were successfully amplified and immobilized demonstrating digital solid-phase PCR. Compared to widely established emulsion based PCR (emPCR) approaches, leading to PCR products immobilized onto bead surfaces in a highly parallel manner, the novel technique results in direct spatial registration of immobilized PCR products in a microarray format. This enables the subsequent use for massively parallel analysis similar to standard microarrays [5].

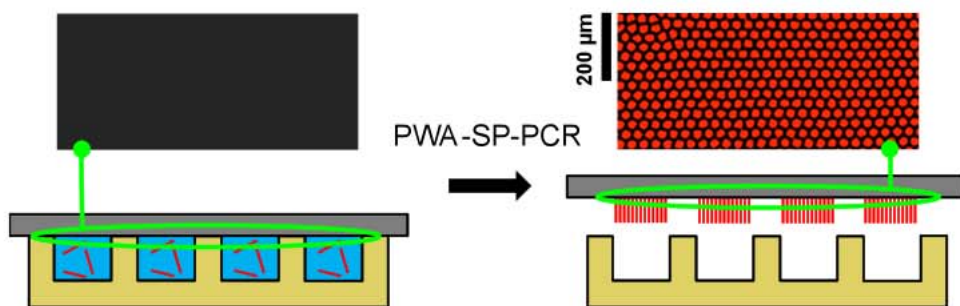


Figure 4: Illustration of highly parallel PCR reactions in a picowell array (PWA) and simultaneously immobilizing the generated PCR products in a covalent and spatially-resolved manner onto a microscope slide covering the PWA via solid-phase PCR (from [5]).

CONCLUSIONS

We expect that the new strategy of designing microfluidic chips as “Apps” for standard instruments could push the market penetration of microfluidic devices. Extending the approach of designing microfluidic Apps will enable end-users to profit from the large potential of microfluidic system integration with low or moderate initial investments for new equipment.

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