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Lab-on-a-chip solutions designed for being operated on standard laboratory instruments

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Abstract

In this paper, we propose the development of microfluidic disposables that can be processed with standard laboratory instruments. The use of prevalent processing devices could significantly reduce existing market entry barriers for lab-on-a-chip solutions and support the market uptake of microfluidic products. We demonstrate the concept with the following applications:

- microfluidic chips for DNA-purification operated on a standard laboratory centrifuge with 42% yield compared to gold standards (QIAamp, Qiagen GmbH)
- microfluidic foil disk for DNA pre-amplification, aliquoting, and real-time PCR operated on a slightly modified Corbett Life Science thermocycler (now Qiagen) with < 10 copy sensitivity
- microfluidic disposable for isothermal DNA amplification by recombinase polymerase amplification also operated on a Corbett Life Science (now Qiagen) thermocycler with < 10 copy sensitivity and a time-to-result of < 15 minutes.
- fully automated hematocrit measurement in a DVD ROM drive from < 10 µL of whole blood.

Keywords: lab automation; process automation; microfluidics; laboratory instruments; DNA purification; PCR, RPA, hematocrit

1. Motivation

Although disposable lab-chips can be produced at low cost, rapid market penetration can be hindered by high initial invests for instrumentation. We propose the paradigm change to use available instruments for processing lab-on-a-chip devices instead of developing tailor-made, expensive new instruments. This reduces initial

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investments for the user to almost zero and allows the microfluidic automation of laboratory protocols at little costs. We expect that this approach will significantly increase the acceptance of lab-chips by the end-user.

2. Application examples

The following examples show microfluidically integrated laboratory protocols that are processed on commercially available instruments. This demonstrates the wide range of applications that can already be realized with microfluidic disposables that are tailored to existing processing instruments and not vice-versa.

2.1. DNA purification on a standard laboratory centrifuge

We demonstrate the microfluidic automation of a solid phase DNA purification protocol in a standard laboratory centrifuge [1]. The microfluidic disposable contains pre-stored liquid reagents in glass ampoules and an integrated silica membrane (Figure 1). After the disposable is inserted, all glass ampoules are crushed and the centrifugation is started. In 3 preliminary experiments, 150±93 ng DNA were extracted from 32 µL lysed whole blood (42% of reference extraction with QIAamp DNA blood mini kit). The only manual steps are to pipette the sample and elution buffer into the disk, crush the ampoules and to close the lid before starting the 2-stage centrifugation.

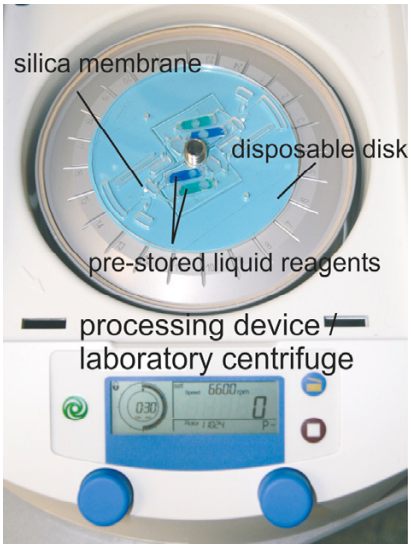

Fig. 1. Microfluidic disposable disk for DNA purification in a standard laboratory centrifuge. The required washing buffers are pre-stored.

2.2. Geometrically multiplexed real-time PCR in a commercial centrifugal thermocycler

DNA analysis is a very attractive yet challenging field for µTAS. We developed microfluidic foil disks [2] that can be processed in an instrument for real-time PCR, the Rotor-Gene (RG) of Corbett Life Science, recently acquired by Qiagen (Figure 2). The RG has been slightly modified to allow access to higher centrifugation frequencies (~27 Hz). As one application example, we developed a microfluidic structure for genotyping assays from a 90 µL sample. The sample is automatically split into 8 cavities with pre-stored primers and probes. Then, individual real-time PCR is performed in each cavity [3]. < 10 DNA copies per cavity could be detected (Figure 3).

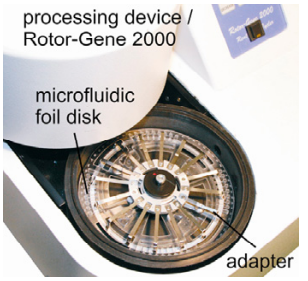

Fig. 2. Microfluidic foil disk in a slightly modified version of a commercially available centrifugal thermocycler. A simple and inexpensive mechanical adapter and a slight adaptation of the device's integrated electronics are the only requirements [3].

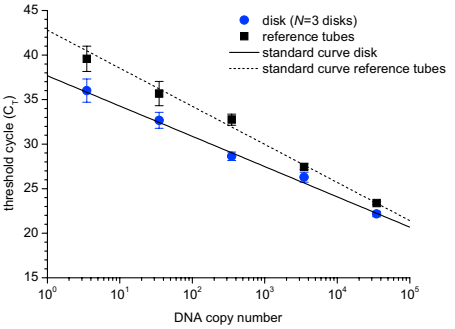

Fig. 3. Comparison between reference PP tubes and foil disk. Shown are threshold cycles of the qPCR according to the applied DNA copy number in the sample. The PCR protocol was the same for the reference and the disk (data according to [3]).

2.3. Geometric multiplexing and reagent distribution for an isothermal polymerase amplification in the Rotor-Gene

Novel approaches also allow isothermal amplification and detection of DNA. We established an integrated microfluidic assay based on the Recombinase Polymerase Amplification (RPA) technology [4]. It allowed detection of the antibiotic resistance gene *mecA* of *Staphylococcus aureus* from ≤ 20 starting copies in < 15 minutes at a constant temperature of 37 °C [5]. All required dry and liquid reagents were pre-stored in the test carrier (Figure 4).

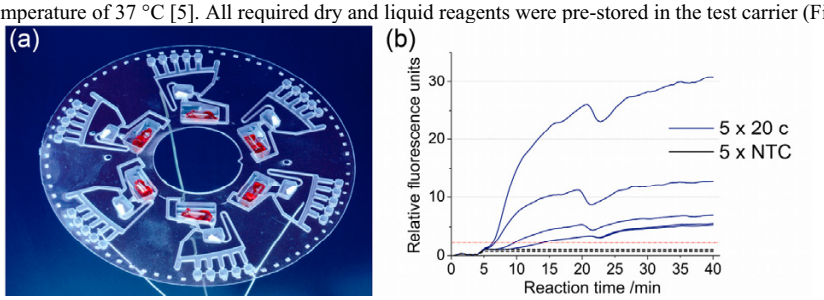

Fig. 4. Microfluidic foil disk for RPA amplification and detection of DNA in the Rotor-Gene. (a) The assay buffer and the dried master-mix are pre-stored in the disposable. (b) Assay curves for 20 starting copies and no template controls (NTC) (figures according to [4]).

2.4. Hematocrit measurement in a DVD drive

The ultimate reason to develop "microfluidic DVDs" for standard DVD-drives is the low cost of the DVD-drives and DVDs. We realized a fully automated hematocrit test (Figure 5) operated in a DVD drive with modified firmware [6;7]. After insertion of 8-10 µL blood, the sedimentation and readout are fully automated by the device.

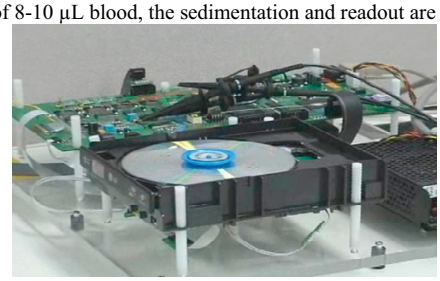

Fig. 5. DVD with microfluidically integrated hematocrit testing structures in DVD drive with modified firmware (figure according to [6]).

3. Conclusions and outlook

We summarized our latest results in microfluidic automation of laboratory processes in standard instruments, including DNA purification, multiplex DNA amplification, and hematocrit measurement. Using common laboratory instruments as processing devices for microfluidic automation has clear benefits in terms of instrument costs and dissemination. Consequently, everybody could benefit from microfluidically automated laboratory protocols without significant investments. In the future this might support the breakthrough of lab-chips in everyday lab situations.

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References

[1] Mark D, Rombach M, Lutz S, Zengerle R, von Stetten F. Microfluidic Unidirectional Pneumatic Switch for Automated DNA-Extraction on Standard Laboratory Centrifuges. *Proceedings of the 13th International Conference on Miniaturized Systems for Chemistry and Life Sciences* (µTAS) 2009;110-2.

[2] Focke M, Kosse D, Müller C, Reinecke H, Zengerle R, von Stetten F. Lab-on-a-Foil: microfluidics on thin and flexible films. *Lab Chip* 2010;**10**:1365-86.

[3] Focke M, Stumpf F, Faltin B, Reith P, Bamarni D, Wadle S *et al*. Microstructuring of polymer films for highly sensitive genotyping by real-time PCR on a centrifugal microfluidic platform. *Lab Chip* 2010 (accepted); DOI:10.1039/C004954A

[4] Piepenburg O, Williams CH, Stemple DL, Armes NA. DNA detection using recombination proteins. *PLoS Biology* 2006;**4**:1115-21.

[5] Lutz S, Weber P, Focke M, Faltin B, Hoffmann J, Müller C *et al*. Microfluidic Lab-on-a-Foil for Nucleic Acid Analysis based on Isothermal Recombinase Polymerase Amplification of DNA. *Lab Chip* 2009;**10**:887-93.

[6] Burger J, Mark D, Roth G, Müller C, Zengerle R, von Stetten F, Park Y. Physiologische Assays in einem DVD Player am Beispiel der Messung des Hämatokrit. *Proceedings of VDE Mikrosystemtechnik-Kongress* 2009;663-5.

[7] Riegger L, Grumann M, Steigert J, Lutz S, Steinert CP, Müller C *et al*. Single-step centrifugal hematocrit determination on a 10-$ processing device. *Biomed Microdevices* 2007;**9**:795-9