Lab on a Chip

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CRITICAL REVIEW

Microfluidic Apps for off-the-shelf instruments

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Within the last decade a huge increase in research activity in microfluidics could be observed. However, despite several commercial success stories, microfluidic chips are still not sold in high numbers in mass markets so far. Here we promote a new concept that could be an alternative approach to commercialization: designing microfluidic chips for existing off-the-shelf instruments. Such "Microfluidic Apps" could significantly lower market entry barriers and provide many advantages: developers of microfluidic chips make use of existing equipment or platforms and do not have to develop instruments from scratch; end-users can profit from microfluidics without the need to invest in new equipment; instrument manufacturers benefit from an expanded customer base due to the new applications that can be implemented in their instruments. Microfluidic Apps could be considered as low-cost disposables which can easily be distributed globally *via* web-shops. Therefore they could be a door-opener for high-volume mass markets.

Microfluidics and μTAS

The original concept of miniaturized total analysis systems (μTAS) was introduced by Andreas Manz *et al.* in 1990.[1] He promoted the integration of processing steps necessary for chemical analysis into a single chip by making use of miniaturization. Nowadays this field of research is also known as microfluidics. In view of currently more than 3000 publications annually,[2] research in the field of μTAS and microfluidics can indeed be regarded as an extraordinary scientific success story. Especially the introduction of poly(dimethylsiloxane) (PDMS) in the late 1990s[3] and the concept of "microfluidic large scale integration" can be considered as milestones for the microfluidics community, since they enabled parallel control over thousands of valves and hundreds of chambers on a single chip with an edge length of only a few centimetres.[4,5]

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Daniel Mark studied physics at the University of Ulm, Germany and the University of Oregon, USA, receiving an MSc degree and German diploma in 2006/2007. In 2007, he started his work as an R&D engineer and PhD candidate at the Department of Microsystems Engineering (IMTEK) of the University of Freiburg, focussing on lab-on-a-chip applications for medical diagnostics. In 2008, he became group leader of the centrifugal microfluidics team of the joint lab-on-a-chip research division of IMTEK and the HSG-IMIT. Since 2011, he is head of the Lab-on-a-Chip Design- & Foundry Service, offering customer-specific lab-on-a-chip solutions. His research experience includes microfluidic design, prototyping, and validation of biomedical applications.

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Dr Felix von Stetten studied Agricultural Engineering and Dairy Sciences at the Technical University of Munich, Germany. After additional studies in biotechnology and a research period in food microbiology he received his PhD in microbiology, also from the Technical University of Munich in 1999. Then he spent three years in the diagnostic industry and was involved in the development of methods for sample preparation, real-time PCR and DNA-arrays. Afterwards he joined the Laboratory for MEMS Applications at IMTEK, University of Freiburg, where he became involved in biofuel cell- and lab-on-a-chip research. Today Felix von Stetten heads the joint research division lab-on-a-chip of IMTEK and HSG-IMIT.

There are numerous examples of μTAS and microfluidic solutions that have been demonstrated. Some already have a large commercial impact such as the Agilent Bioanalyzer (http://www.genomics.agilent.com) for sizing, quantification and quality control of DNA, RNA, proteins and cells, the Cepheid GeneXpert (www.cepheid.com) for molecular analysis, or the Fluidigm systems for highly multiplexed PCR (www.fluidigm.com). Often overlooked mass markets for microfluidics exist as well, such as test strips for *e.g.* pregnancy tests or cartridges for ink-jet printers. Many more exciting microfluidic solutions have been demonstrated in academia, such as the isolation of circulating tumor cells[6] or fully integrated multi-parameter immunoassays for rapid point-of-care testing.[7] These examples underline that the promise of microfluidics and μTAS has certainly lost nothing of its charm. This is also reflected by a substantial microfluidics market, which is expected to reach almost $2 billion in 2012.[8]

However, compared to the research effort invested, there is only a limited number of end-user products to date.[8] Especially when being compared to the standard MEMS market in which sensor-chips such as accelerometers, gyros or pressure sensors are manufactured in hundreds of millions of units per year; up to now there is no high volume chip application in the field of microfluidics. One of the most successful products on the market, the Cepheid GeneXpert, seems to currently feed a global market of less than 1 million tests per year (http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/xpert_mtb_rif.html, accessed November 2011). This indicates still remaining significant market entry barriers which must be overcome before a commercial breakthrough can be achieved.

Market entry barriers

Market penetration is hindered by technical as well as non-technical aspects. Assuming the technical hurdles such as accuracy, reproducibility, long-term stability, *etc.* can be overcome with the current effort invested into microfluidics research worldwide, non-technical aspects are put into focus. First of all, microfluidics in itself is no product but an enabling technology. Especially one lesson was learned in the often cited "trough of disillusionment" of the Gartner Hype Cycle Model that microfluidics went through in the 2000s:[9,10] the market expects microfluidics to solve specific problems in analytics with affordable solutions, and will not accept it as self-purpose. Currently, developments that include microfluidic components are often time consuming and expensive developments not only for the microfluidic chip but also for the processing instrument. Additional issues are high overall development risks and the required clearances if products are developed for regulated markets. All those factors add up to high costs and it is a challenge to identify applications having a good potential of recovering such high investments.[11] And once these hurdles have been taken, the customer is confronted with significant initial investments for a lab-on-a-chip processing instrument which usually is in the range of $10 000–$100 000 or even more just for running a first chip.

Microfluidic Apps – the shortcut to market for microfluidics?

Now imagine inexpensive disposables which greatly enhance the application range for instruments already available in the labs or at home. Imagine that your laboratory centrifuge can now be used as an automated workstation for DNA purification or your DVD drive for chemical sensing. Microfluidic companies would not have to spend money on developing instruments, building up sales networks and could even save the costs for maintenance of instruments. End-users would not be discouraged by high investments for an instrument just to be able to run a first chip. The customers would profit from miniaturization, integration and automation of liquid handling operations without even knowing that there is microfluidics inside. Lower development costs for microfluidic companies would reduce the threshold for the return-of-investment and due to that a broad range of new applications would become economically feasible. And finally, if those instruments are already available in the labs or at home, the suppliers of those instruments could profit from a largely expanded customer base due to numerous new applications being enabled.

This article will show that this scenario is not only a vision but is already becoming reality. Microfluidic chips can be designed to be operated on existing instruments that are already common in labs or at home. This approach could be called "Microfluidic Apps" for off-the-shelf instruments. The approach is very similar to the concept of Apps for smartphones: Apps are inexpensively developed and easily distributable software products brought to the market *via* the internet on a global scale. As a consequence suppliers of smartphones can sell their product to a huge customer base. In this analogy the microfluidic chip corresponds to the software App and the processing instrument to the smartphone (Fig. 1). The idea of "Apps" proved to be a winner for smartphones and could be the same for microfluidics.

However when considering commercialization and staying in this analogy, untested Apps for smartphones pose the risk of jeopardizing the functionality or integrity of the device. Some companies such as Apple solve this issue by only allowing approved Apps to be installed. Others, such as the Android market, leave that risk but also that freedom of choice to the customer. In general, the answer whether only a closely

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Fig. 1 Apps greatly enriched the possibilities of smartphones. In terms of automated analysis, Microfluidic Apps could do the same for existing laboratory hardware.

controlled and approved selection of Microfluidic Apps are accepted by instrument manufacturers or if open platforms without controls emerge, will most likely depend heavily on the application targeted by the Microfluidic App. For heavily regulated markets such as diagnostics, developers of Microfluidic Apps and instrument builders would have to work closely together and define and share approval procedures, responsibilities and warranties. For academic research, *e.g.* the automatic generation of dilution series on centrifuges, it is conceivable that instrument builders allow the use of a large range of Microfluidic Apps without the loss of warranty, since the range of applications for the instrument and thus its customer base are expanded and the instrument builders benefit by larger sales numbers, while the developers of Microfluidic Apps generate turnover by selling the disposables. In any case, instrument builders will definitely have to be involved to some degree and give their consent for Microfluidic Apps. In our experience though, instrument builders are very open and interested in such collaborations.

Microfluidic Apps – a survey of recent publications

Developing a Microfluidic App starts with the identification of the need of a certain user-group and the fluidic protocol for implementing it. As a next step the instruments available in the environment of the users as well as the capabilities of those instruments have to be analyzed. The final task is to develop the microfluidic chip that implements the fluidic protocol harnessing the capabilities of the instrument (Fig. 2). In the following we list Microfluidic Apps that were developed for a given instrument or class of instruments.

Microfluidic Apps for glucose meters

Glucose meters are handheld point of care devices for home diagnostics. Fluidic protocols are limited to purely capillary driven mechanisms, but various sub-types of existing glucose meters offer optical or electrical read-out capabilities. Whitesides' group developed a Microfluidic App for clinical chemistry, read out by a standard electrochemical glucometer.[12] The microfluidic device is made out of paper and in addition to the quantification of glucose also measurement of cholesterol and L-lactate in human plasma was demonstrated. Benefits of this system include an extremely low-cost disposable chip running on an off-the-shelf electrochemical glucose reader available for less than $20.[12] Microfluidic Apps for glucose meters are especially of interest for developing countries which can profit from integrated and affordable point-of-care tests.[13,14] Above that the Whitesides group also demonstrated the separation of plasma from whole blood by utilizing a hand-powered egg beater.[15]

A qualitative cost-benefit analysis of this approach is quite favourable for the Microfluidic App due to the very low quoted costs and especially the lack of alternative diagnostic tools in low-resource settings. Considering that this approach could deliver basic diagnostic capabilities to remote areas with little infrastructure and do this at an affordable price also for developing countries, it seems like a very attractive solution.

Microfluidic Apps for optical disc drives

Optical disc drives such as CD-, DVD- or Blu-ray drives have a rotary motor, a laser based light source and a photo-detector which can be used for processing and read-out of a microfluidic chip. They are very appealing instruments for Microfluidic Apps since they are very inexpensive ($10–$50) and available in the labs as well as at home. A number of Microfluidic Apps for optical disk drives were published including automated hematocrit measurements,[16,17] Ca^{2+}-concentration measurements,[18] biotin/streptavidin binding, DNA hybridization and IgG/anti-IgG interactions[19] and an immunoassay for C-reactive protein (CRP).[20] However, most of the presented examples still require extensive manual preparation steps and are not plug-and-play solutions yet. Still, in our opinion it should be possible to develop fully integrated Microfluidic Apps for optical drives *e.g.* performing absorption based assays for clinical chemistry or even homogeneous immunoassays for home based diagnostics.

Microfluidic Apps for laboratory centrifuges

Instruments such as optical disk drives and glucose meters are inexpensive and potentially available everywhere but of limited capabilities in terms of liquid processing, thermal control and optical readout. In contrast, modern laboratories feature a large variety of standard instruments which offer highly precise optical, thermal and mechanical control elements. Among those, laboratory centrifuges are available in almost every lab. They feature control over frequency of rotation up to thousands of *g*, some allow temperature control, or are programmable *via* an external computer or *via* a user interface. A Microfluidic App for the automation of DNA-purification from lysed blood on a standard laboratory centrifuge was shown by Müller *et al.*[21] All the liquid buffers were pre-stored on the microfluidic chip and the automated fluidic protocol constitutes the sequential release of the buffers, the binding, two washing steps, the elution as well as the routing of the liquids.[21] Another Microfluidic App was developed for automated generation of dilution series of 1 : 3 and 1 : 5 on standard lab centrifuges.[22] Several issues including

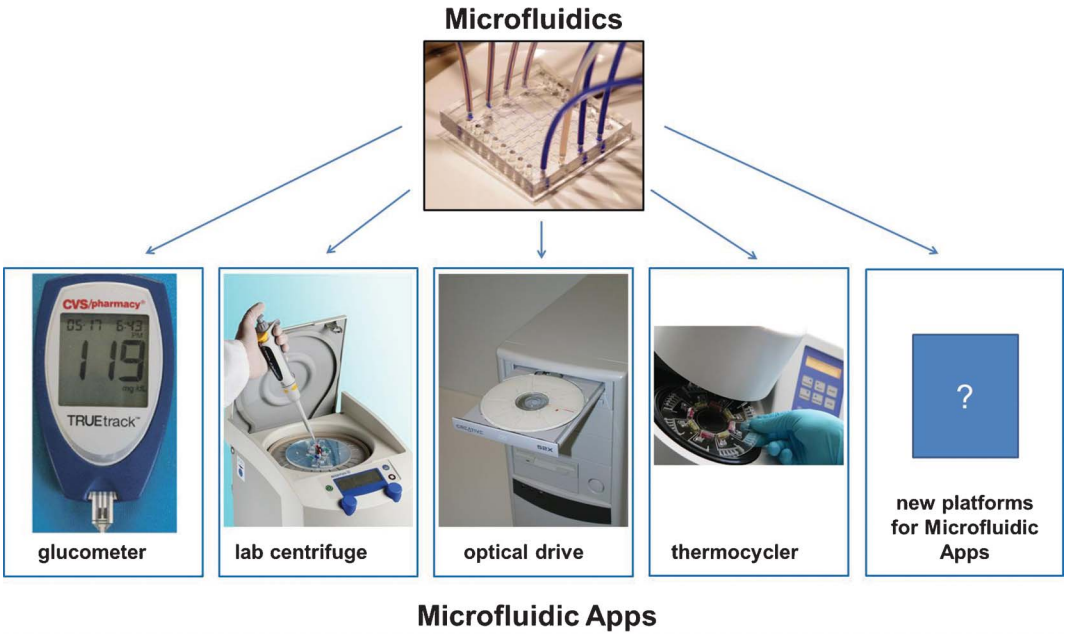

Fig. 2 From microfluidics to "Microfluidic Apps". Microfluidics promises automation and miniaturization of biochemical assays. The next step towards higher end-user acceptance and impact could involve the adaptation of microfluidic liquid handling to fit standard instruments, such as glucometers, centrifuges, optical drives or thermocyclers. The depicted examples of such "Microfluidic Apps" for standard hardware could also help to decrease development risks and time for microfluidic applications.

performance compared to reference methods, production costs and shelf-life have still to be evaluated in detail. Nevertheless, automation of standard laboratory protocols replaces tedious manual handling steps, enable "start and walk away" processing and are of high value for personnel working in the lab. Due to the fact that those Apps target applications in non-regulated markets and automate everyday lab tasks, they could be an enabler for high volume markets of microfluidic chips.

Microfluidic Apps for rotary PCR thermocyclers

With more complex instruments, also more demanding assays and protocols can be implemented. Rotary PCR thermocyclers feature control over frequency of rotation, temperature, integrated light sources for excitation as well as various filters and highly sensitive detectors for fluorescence read-out. Focke *et al.* developed a Microfluidic App for genotyping of sub-types of methicillin-resistant *Staphylococcus aureus* (MRSA),[23] potentially addressing a pressing concern in the increasing awareness of hospital acquired diseases. Starting from a purified DNA sample, the automated assay protocol constitutes a 10 cycle pre-amplification, diluting and mixing, 14 fold aliquoting, another mixing and incubation step as well as a secondary real-time PCR including read-out. The Microfluidic App contains a disposable chip that is used together with a rotor replacing the original sample wheel with reaction tubes of the instrument. The demonstrator still relies on external sample preparation but as mentioned before there would be other Apps available performing such preparation on a standard centrifuge.

Since this Microfluidic App is very familiar to the authors, it will be used for an exemplary quantitative cost–benefit analysis, demonstrating that Microfluidic Apps have the potential to be commercially competitive also in a high-tech environment. Realistically, we will assume a small-scale market of initially 1000s of disposables and a simplified layout without pre-amplification. Offers from injection molders quote sales prices of about 4.50 € per plastic disposable for a batch of 1000 without reagents or filling. Filling of the reagents and sealing could initially amount to the same costs, resulting in roughly a 9 € sales price per disposable when produced in small numbers. Considering the economy of scale[24] for hot embossing and according to our estimates, this number could be halved (4.50 €) for batches of 10 000s or more and probably be reduced to 2 € or less for production in the 100 000s. The purely financial benefit amounts to the reduced labour time of a skilled technician in a high-tech laboratory, assumed to be roughly 6 min for mixing the template with the mastermix, aliquoting in 10 reaction containers, mixing with primers and probes and starting the run. With a Microfluidic App, this time would be reduced to 1 min. Assuming costs of 60 € h^{-1} including overhead, the saved 5 min would save 5 € and thus be in the same order of magnitude as the costs for the disposable. This is in itself hardly a selling point and serves only to show that Microfluidic Apps do generate a certain amount of running costs that have to be balanced against the time they save. However, when factoring in that in this case the Microfluidic App would also greatly reduce the risk of pipetting errors and contaminations and lead to a much more convenient workflow, there would be a real benefit for the end-user, especially where high reproducibility is essential. In summary, the cost–benefit evaluation for each application will certainly determine which Microfluidic Apps have a chance on the market.

Microfluidic Apps for standard optical readers

This section will briefly summarize other solutions which can be considered "Microfluidic Apps", since they utilize microfluidic

phenomena to implement new functions on existing instrument platforms.

Siloam Biosciences, Inc. integrated microfluidics in a microwell plate for improved processing of immunoassays (https://www.siloambio.com). The modified microwell plate can be regarded as a Microfluidic App for standard plate readers. Due to integrated microfluidics and the favorable reaction kinetics the sample volume can be reduced to 5 μL corresponding to a 20 fold reduction compared to standard assays without loss of sensitivity.

Another application example is the fast serial readout of encoded gel microparticles in standard flow cytometers by Firefly Bioworks (www.fireflybio.com).[25] The proprietary microparticles *e.g.* allow quantification of microRNA by hybridizing a sample to mixed coded microbeads which contain different complementary microRNA probes. Subsequent fluorescent labeling allows identification of the codes on the microparticles and quantification of the different microRNA levels by fluorescence readout.

Future prospects

Numerous examples of Microfluidic Apps running on standard instruments have been demonstrated, ranging from automated DNA purification on a standard centrifuge to immunoassays on a CD-ROM drive. Of course, many challenges remain: for instance, many Microfluidic Apps require at least a firmware update of the commercialized instruments, leading to additional efforts over just using an existing instrument. Nonetheless, the required resources are substantially less then developing an instrument from scratch.

Designing Microfluidic Apps for standard instruments should be appealing to microfluidic developers as well as to builders of instruments and manufacturers of polymer disposables. In order to support market entry and to pool know-how, providers of microfluidic solutions, vendors of promising commercialized devices and cartridge manufacturers could embark on joint developments for a joint benefit. The symbiosis of microfluidic liquid handling and commercially successful processing instruments could open new market opportunities on the one hand and spearhead the commercial dissemination of useful microfluidic products on the other hand. All parties would profit, while the end-user gains immediate access to Microfluidic Apps which facilitate lab work, enable new analytical applications or even allow home diagnostics with almost no investments in instruments. All that is required are researchers realizing the potential of Microfluidic Apps that are designed to fit existing instruments from household, healthcare or laboratory, just as programmers quickly realized the benefits of smartphone platforms by contributing a wealth of new applications.

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