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Microfluidics: An Enabling Technology for the Life Sciences

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ABSTRACT

During the last year we have investigated existing and future markets, products and technologies for microfluidics in the life sciences. Within this paper we present some of the findings and discuss a major trend identified within this project: the development of microfluidic platforms for flexible design of application specific integrated microfluidic systems. We discuss two platforms in detail which are currently under development in our lab: microfluidics on a rotating CD ("Lab-CD") as well as a platform to realized customized "nanoliter & picoliter dispensing systems".

1. INTRODUCTION

In the course of the EU-sponsored "FlowMap" project, a consortium of strong partners comprising IMTEK (D), HSG-IMIT (D), Cranfield Biotechnology Center (UK) and Yole Développement (F) has analyzed existing and future markets, products and technologies for microfluidics in the life sciences. During this one-year project, more than 150 external experts have been involved in creating a microfluidics roadmap by a series of designated workshops, personal interviews and a world-wide questionnaire action [1]. As a result, we have quantified the economic development and pinpointed important market drivers. Furthermore, the paramount technology drivers which will determine the present and expected capabilities have been identified.

Regarding the economical impact of microfluidics the majority of experts expect an overall growth rate for microfluidic technologies and products in the life sciences of more than 30% per annum with

- drug discovery
- medical diagnostics and
- therapeutic devices

representing the most promising fields. In a systematic market analysis based on the data acquired during the FlowMap project, our partner Yole Développement has estimated the global market of microfluidics in the life sciences to approximately 500 M€, increasing with an assumed annual growth rate of 19% to 1.4 billion € in 2008. The FlowMap report [1] presents a detailed breakdown of this turnover in each microfluidics segment identified within the Life Sciences.

Besides the economical impact of microfluidics also technological trends have been clearly pinpointed within the roadmap. Among those are

- the need for validated and easy to operate microfluidic platforms which give the users the freedom to easily combine the basic modules for different fluidic operations in order to build application specific microfluidic systems
- the use of low-cost technologies such as injection moulding or low-cost substrates such as PDMS (polydimethylsiloxane), PCB or Polyimide for the fabrication of microfluidic devices

2. WHY DO WE NEED MICROFLUIDIC PLATFORMS?

When you go to conferences these days – no matter whether they are Life Science oriented conferences or MEMS oriented conferences – "microfluidics" is one of the most popular and the most referenced key words in the contributions. This is quite amazing because microfluidics is nothing what you want to buy as a consumer. In most cases microfluidics is just a toolbox, which is needed to develop innovative products for a diversity of fields like medical, pharmaceutical and analytical applications. Due to that the most important customer for microfluidics know-how and technologies is the research community itself developing new products in the different application areas such as the biotechnological, diagnostic, medical or pharmaceutical industry.

In the last two decades thousands of researchers eagerly sought to develop new microfluidic components or to explore the basic microfluidic operations such as fluid transport, fluid metering, fluid mixing, valving or concentration and separation of molecules within miniaturized quantities of fluids. Nowadays we know hundreds of different types of micropumps [2], hundreds of different types of mixers [3] and hundreds of different types of microvalves. Do we still need to keep on extending the already existing plethora of devices and functional principles before we start to explore the huge potential of different applications in the life sciences? Not really! We expect that the described proceeding represents the past and we do not expect that this will continue for the future. What the research community in the different areas of application really needs are, in our view, validated and easy to operate microfluidic platforms.

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3. WHAT IS A MICROFLUIDIC PLATFORM?

Very similar to the ASIC industry in microelectronics, a dedicated microfluidic platform has to contain a reduced set of validated elements to perform the basic fluidic operations within an given application area. Such basic fluidic operations are e.g. fluid transport, fluid metering, fluid mixing, valving and separation or concentration of molecules or particles. The bundle of fluidic operations needed for diagnostic applications will be different compared to the bundle needed for pharmaceutical applications. Thus in some cases also detection methods will belong to the basic set of microfluidic operations and in other cases not (table 1). Nevertheless in all cases the user of such a platform has to be able to easily combine the basic modules within a given platform in order to implement an assay for diagnostic applications or to screen for new drug compounds in pharmaceutical applications.

Most importantl, all modules of a given microfluidic platform have to be easy to fabricate within a well defined and well controlled technology. And last but not least, all modules of a platform have to be connectible, ideally in a monolithic way, or at least by a well defined and easy packaging technique. If a platform allows a seamless integration of different fluidic modules in a monolithic way, e.g. without sophisticated additional packaging techniques, this will be a significant advantage compared to other platforms.

Microfluidic Operations	**Fabrication Technology**
validated modules for basic microfluidic operations such as o fluid transport o fluid metering o fluid valving o fluid mixing o separation o concentration o detection o …	validated manufacturing technology for the whole set of fluidic modules
	seamless integration of different modules o ideally in a monolithic way o or by a well defined and easy packaging technique

Table 1: Common features of microfluidics platforms.

4. EXAMPLES OF MICROFLUIDIC PLATFORMS

The spatial limitations of this paper does not allow to give a complete overview of the microfluidic platforms currently under development in the research community. Therefore the succeeding two examples which are explored in our lab just give a first impression regarding the big potential of the platform concept in general.

4.1 Microfluidics on a Rotating CD ("Lab-on-a-Disk")

The "Lab-on-a-Disk" platform is inspired from the conventional compact disk (CD) known from the IT-industry [10], [11], [12], [13], [14]. It contains on the one hand a disposable disk integrating various passive microfluidic components like channels and mixing chambers and on the other hand an equipment similar to an ordinary CD player which controls the rotational frequency of the Lab-on-a-Disk (Fig. 1). As will be shown in the next section, the platform allows a seamless and cost effective integration of the various microfluidic operations needed in lab-on-a-chip applications [13].

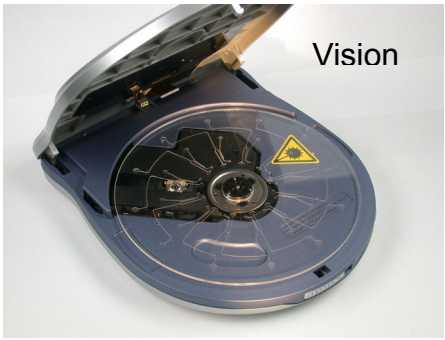

Fig. 1: Future vision of the Lab-CD platform: a portable player combined with an application specific, disposable disk [14].

Liquid samples are introduced into the microfluidic channels near the centre of the disk. As the disk rotates the liquid is transported by centrifugal forces to the periphery of the disk. The flow type created by centrifugal forces is a continuous pulse-free which gives also some additional advantages for the various applications. The liquid flow can be stopped on a rotating disk by capillary burst valves that provide valving without moving actuation elements [10]. Capillary valving is based on either a hydrophobic barrier or an abrupt change in the cross-sectional channel geometry. In both cases an effective capillary pressure at the barrier counteracts the centrifugal pressure induced on the liquid plug. The burst frequency for a particular valve structure, at which the centrifugal pressure exceeds the capillary pressure, is defined by a set of impact parameters which can be controlled at the disk fabrication. Metering of liquids can easily be done by chambers containing a well defined volume. The outlet of such a chamber is blocked by a capillary burst valve and the excess liquid is purged via drain channels into a waste chamber. Mixing and switching of different reagents have been demonstrated in various ways [15], [16]. Last but not least the Lab-on-a-Disk platform can be regarded as a centrifuge which can be used in an elegant way to separate particles

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from liquid matrices, e.g. red blood cells from blood plasma as depicted in Fig. 2 [17].

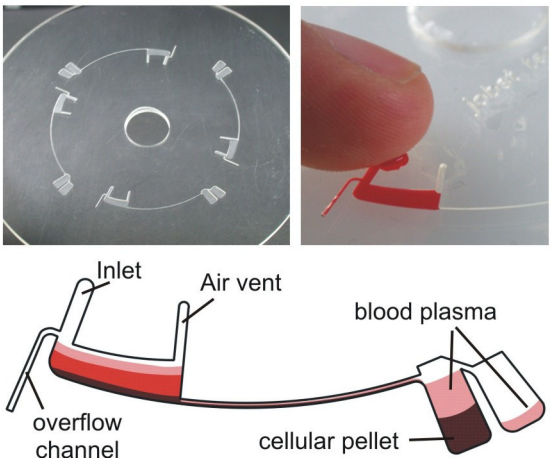

Fig. 2: Structures for fast separation of blood plasma from blood cells within 20 s on a lab-on-a-disk platform [27].

On one Lab-on-a-Disk a multitude of samples can be processed in parallel as the format offers a rather big area compared to microchips which makes it very efficient. There are two companies that have developed lab-on-a-disk systems so far. Gyros in Uppsala, Sweden, performs protein sample preparation on-disk for matrix-assisted laser desorption and ionization mass spectrometry (MALDI-MS). The "Gyrolab" disk processes 96 protein samples simultaneously concentrating and purifying them and in the end crystallizes the proteins directly on the disk. Then the disk is transferred into an ordinary mass spectrometer for detection. A second type of disk is specialized to quantify nanoliters of protein sample with a fluorescence reader integrated into the CD-player-like "Gyrolab Workstation".

The LabCD of Tecan is used for drug discovery. The functionality of newly developed drug-candidates is tested on this disk. Therefore blood serum is mixed with fluorescent probe molecules. These bind to serum proteins giving a fluorescence signal. When drug molecules with the desired functionality are added they substitute the protein-bound fluorescent probe molecules and the fluorescence signal changes. Thus the interactions between different drugs as well as serum protein bindings are investigated.

Within our Bio-Disk project we develop immuno-diagnostic assays of human whole blood. Two exemplary applications are contemplated:

- The determination of the immune status for the 10 most important pathogens such as Tetanus, Diphtheria and Measles directly from a small amount of human blood.
- The time-critical measurement of important blood parameters for emergency care where often only few minutes are crucial for the patient´s convalescence.

Functionalized beads, which are polymer spheres of about 50µm in diameter, are used as sensors. Compared to flat surfaces, this particle based concept offer extraordinary large surfaces, slow diffusion lengths and thus drastically shortened times for affinity reactions with target-molecules. The affinity is detected by a final fluorescence read-out step.

A basic advantage of the Lab-on-a-Disk platform is the control and automation of all fluidic processes by a simple parameter, the rotational frequency of the player. Furthermore, this platforms offers a great potential for cost-effective miniaturisation of portable analysis tools.

4.2 Non-contact nanoliter & picoliter dispensing

A second platform under development in our lab is a microfluidics toolbox for highly parallel non-contact liquid dispensing. It seamlessly covers the volume range from several tens of picoliters to several microliters. A key feature of this platform is the fact that a multitude of individual dispensing units can be easily arranged on a flat substrate in parallel. The platform therefore easily allows to handle hundreds and even thousands of different liquids simultaneously (Fig. 3) at a pitch ranging from several hundreds of microns to several millimetres. The liquids are delivered as free flying droplets or jets in a non-contact manner.

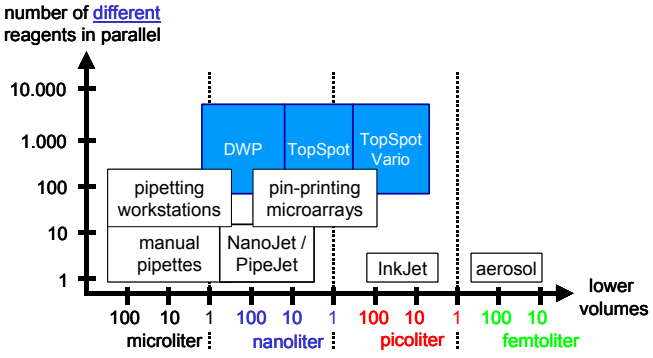

Fig. 3: Map of low volume dispensing. The platform relies on three different techniques of creating well-defined liquid volumes which can be dispensed in a contact-free manner (DWP, TopSpot, TopSpot Vario). The techniques allow to seamlessly cover the volume range from several tens of picoliters to several microliters. Several hundreds to several thousands of dispensing channels can be easily arranged in parallel on a flat substrate allowing to dispense the same amount of different liquids simultaneously.

Though the principle task of generating free flying liquid droplets seems to be quite simple, it is a big challenge to guarantee that the dispensing systems operate for the full

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range of liquids usually covering a certain range of liquid properties (viscosity, surface tension, …) within a given application area. This is obviously not a trivial task as the numerous – still ongoing – publications in the field show. Within our platform we developed three basic dispensing techniques to cover the full volume range from several tens of picoliters to several microliters. The techniques are called DWP, TopSpot and TopSpot Vario.

For all three techniques one single dispensing unit simply consists of a reservoir, a nozzle (nozzle chamber and nozzle orifice) as well as a connection channel. In Fig. 4 the working principles of all three techniques are illustrated for just one single dispensing channel.

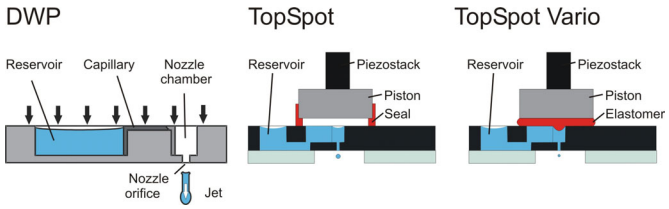

Fig 4: (a) Pressure based actuation principle for dispensing from 10 nl to several µl based on DWP technology; (b) Pressure based actuation for dispensing volumes in the lower nl volume range based on the TopSpot principle; (c) Direct displacement based actuation via an elastomer for dispensing volumes in the 50 pl to 1.000 pl range based on the TopSpot Vario principle

- DWP Dispensing Technology

In the so called DWP™ technology the dispensed volume is defined by the volume of a micromachined nozzle chamber. When liquid is filled into a reservoir, this liquid is transported by capillary forces to the micro nozzle which comprises of a defined geometrical volume. The total liquid contained in the nozzle can then be dispensed by applying a pneumatic pressure pulse. The amplitude and duration of this pressure pulse are in the order of 0.2 bar and 10 ms, respectively. The dispensed volume is defined essentially by the geometrical volume of the nozzle. To a great extent it is independent of the duration and amplitude of the applied pressure pulse [4], [5]. Furthermore it is hardly affected by the liquid properties like viscosity, density and surface tension which makes the method very robust in addition.

- TopSpot Dispensing Technology

In the **TopSpot®** principle a pneumatic pressure pulse on the sub-ms time scale is used to eject the liquid [6], [7]. For this in the back of the nozzle a closed cavity is created which can be compressed on the sub-ms time scale by a piston driven by a piezo-stack actuator. This piston movement generates a pressure pulse in the air chamber. This pressure pulse works on the back of the nozzle, causing (with sufficient dynamics) the nozzle to eject a droplet. The volume of these droplets is typically 1 nL. Other volumes can be achieved by using different nozzle diameters.

- TopSpot Vario Dispensing Technology

The **TopSpot® Vario** principle uses the direct displacement of an incompressible but easy deformable elastomer instead of the compressible air chamber [8], [9]. Movement of the piston causes the elastomer to be displaced into the nozzles. Hereby a kind of micro syringe is formed over the nozzle. A well defined volume of liquid in the nozzle is displaced and therefore ejected from the nozzle. In this direct displacement it is possible to set the ejection volume and the ejection speed by the stroke and speed of the piezo actuator. The achievable volume range is between 100pL and 1400pL (1.4nL).

Common for all three dispensing techniques is the fact that a multitude of dispensing channels can be easily arranged in parallel on a flat substrate as will be shown in the following examples adopted for different applications. Doing this the pitch of the reservoirs can be designed independently from the pitch of the nozzles. This allows to choose the pitch of the reservoirs, which usually is designed in order to allow easy filling by pipetting robots independently from the pitch of the nozzles which usually are arranged in a pitch which is needed for the application (e.g. a few hundreds of µm for the microarray fabrication).

Also common for all dispensing techniques is the fact that the basic structures (reservoirs, capillary channel, nozzle chamber and nozzle orifice) can be fabricated by different technologies in different materials. This has been demonstrated for DRIE-etching of silicon as well as hot embossing and injection moulding of plastics. In the following we describe various applications realized so far.

"Dispensing Well Plate" for "High-Throughput-Screening"

For speeding up liquid handling in High-Throughput-Screening (HTS) all liquids have to be dispensed simultaneously into the wells of standard well plates. The pitch of the reservoirs is 9.0 mm, 4.5 mm or 2.25 mm according to the 96, 384 or 1.536 SBS well plate standard. Fig. 5 shows a 384 channel Dispensing Well Plate based on the DWP technology and especially designed for High-Throughput-Screening in drug discovery. The plate size is 80x120 mm and 16x24 dispensing channels are arranged in parallel at a pitch of 2.25 mm corresponding to the SBS 384 well plate standard. The dispensing volume of every channel has designed to be 50 nl. By applying a pressure pulse to the whole upper surface of the Dispensing Well Plate the dedicated liquid volumes contained in the nozzle chambers are driven out completely. After switching off the driving pressure, the nozzles refill again from the reservoirs

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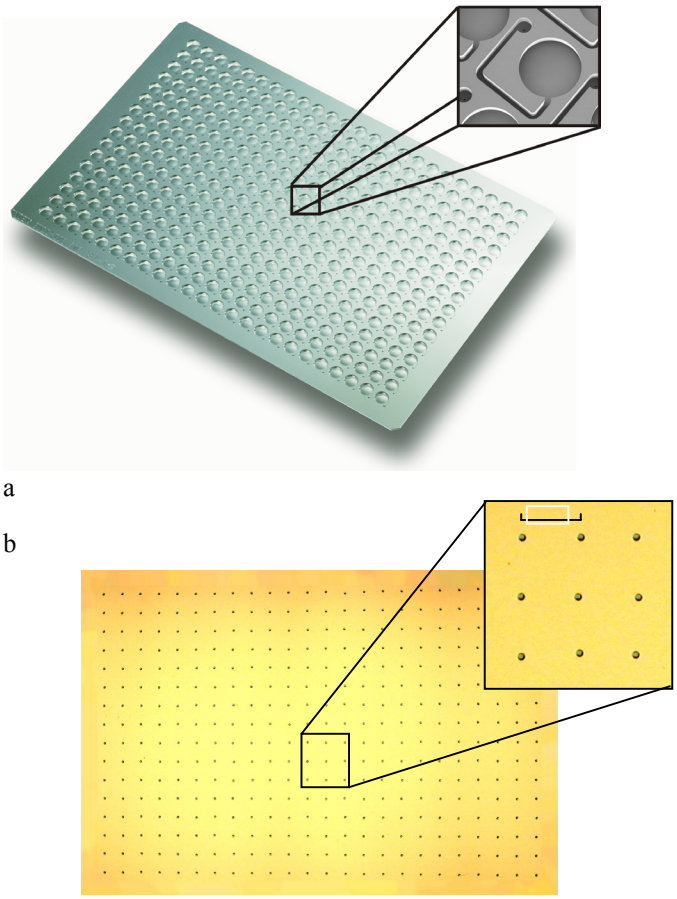
by capillary forces. If the dimensions of the nozzle and the connection channel are properly chosen, the dispensed volume is essentially defined by the geometrical volume of the nozzle which is typically in the range of 50 - 100 nL. If required the nozzle volume can even be raised to several microliters.



a

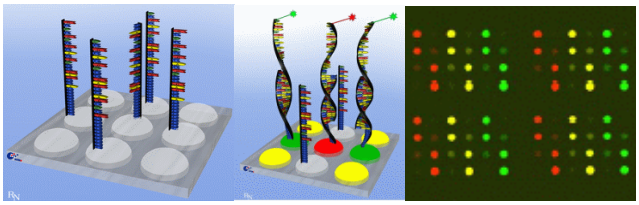
b

Fig. 5: (a) Dispensing Well Plate in 384-format micromachined in silicon; (b) Array of 50 nL droplets simultaneously dispensed by the 384 channel dispensing well plate (pitch: 2.25 mm).

Fluorescence measurements with a plate reader have shown that the homogeneity over all 384 dispensing units coming out of a Dispensing Well Plate micromachined in silicon is within a coefficient of variation (CV) of 5% [4]. Gravimetrical measurements show that the volumetric accuracy can be kept for up to 45 dispensing cycles without a change of dispensing performance. Because of the geometrical definition of the dispensed volume, the dispensed volumes have proven to be independent of liquid properties and actuation parameters within a certain range.

"TopSpot printheads" for
"High-Throughput-Fabrication of Microarrays"

Microarrays are highly parallel biosensors. Their sensor effect is based on a chemical reaction between molecules with a lock and key principle. When producing a microarray, the different catching molecules have to be put at defined pitch (typically 500 µm) on the substrate. The reaction between the catching molecules and a complex mixture of molecules is the actual 'measurement'. Caught molecules can be detected after washing off any surplus material. Microarrays are usually analysed using fluorescence techniques. The molecules in the complex mixture are 'equipped' with fluorescence molecules for later detection.



a b c

Fig 6: Illustration of the working principle of a microarray: (a) probe molecules are immobilized at a regular pitch on a flat substrate; (b) sample molecules hybridise to probe molecules if chemical structures are complementary; (c) fluorescent readout of the microarray

In the detection equipment (scanner) an image is made of the fluorescence signal intensity, which is translated in a characteristic fluorescence image. The position of the catching molecule in its array gives information of the identity of the caught molecule.

The key to enhance the speed and throughput in microarray fabrication is an integrated format in the dispensing printhead. In a TopSpot printhead the reservoirs are arranged at the pitch of 2.25 mm and connected to nozzles typically at a pitch of 500 µm. The liquid is transported simply by capillary forces to the central nozzle area (Fig. 7). Liquid volumes of several µl can be loaded to the reservoirs, enough for dispensing several thousand times without the need to reload the printhead. Figure 8 shows a 96 channel TopSpot printhead.

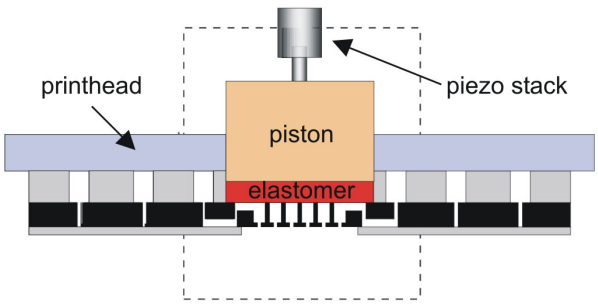


Fig. 7: Schematic layout of the TopSpot Vario printhead [8], [9]. Outer reservoirs are connected nozzles in the centre.

In extensive experiments the TopSpot printheads have been tested for various kinds of liquids and the coefficient of

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variations (CVs) of generated spot diameters were measured to be smaller than 1 % within one single dispensing nozzle and smaller than 1.5 % within all nozzles of a printhead for all used printing buffers [18]. No carry-over and no cross-talk was found in extensive experiments with oligonucleotides and optimized printing buffer compositions and concentrations have been identified.

Fig. 8: TopSpot printhead with 96 dispensing units in parallel. The reservoirs pitch is 2.25 mm, the pitch of the nozzles in the centre area is 500 µm.

5. CONCLUSIONS

Microfluidic platforms will have a huge impact on the development of application specific, integrated microfluidic systems in the future. Such systems can be built very easily based on validated elements of a given platform without the necessity to start always from scratch for nearly every new application as it is still often the case today. Therefore the existence of microfluidic platforms will allow the microfluidics community to leave the device oriented research of today in order to enter into the next challenge: the flexible and cost efficient development of hundreds of different applications accessible by using the full potential of microfluidics and by following a system oriented approach.

REFERENCES

[1] „FlowMap - Microfluidics Roadmap for the Life Sciences"; edited by Jens Ducrée and Roland Zengerle; 2004; ISBN code: 3-8334-0744-1; 197 pages; available via www.microfluidics-roadmap.com

[2] D.J. Laser and J.G. Santiago; "A Review of Micropumps"; Journal of Micromechanics and Microengineering; 14, 2004, R35 – R64

[3] V. Hessel, S. Hardt, H. Löwe, "Chemical Micro Process Engineering: "Fundamentals, Modelling and Reactions"; 2004; ISBN 3-527-30741-9 - Wiley-VCH, Weinheim

[4] Peter Koltay, Reinhard Steger, Benjamin Bohl, Roland Zengerle; "The Dispensing Well Plate: A Novel Nanodispenser for the Multi Parallel Delivery of Liquids (DWP Part I)"; Sensors & Actuators A; available online since 28.07.2004

[5] Peter Koltay, Jan Kalix, Roland Zengerle; "Theoretical Evaluation of the Dispensing Well Plate Method (DWP part II)"; Sensors & Actuators A; available online since 27.07.2004

[6] O. Gutmann, R. Kuehlewein, S. Reinbold, R. Niekrawietz, C. P. Steinert, B. de Heij, R. Zengerle, M. Daub; "A Highly Parallel Nanoliter Dispenser for Microarray Fabrication"; Biomedical Microdevices 6:2, 131 – 137, 2004

[7] O. Gutmann, R. Niekrawietz, R. Kuehlewein, C.P. Steinert, B. de Heij, R. Zengerle, M. Daub; "Impact of Medium Properties on Droplet Release in a Highly Parallel Nanoliter Dispenser"; Sensors & Actuators A; available online since 04.06.2004

[8] B. de Heij, C. Steinert, H. Sandmaier, R. Zengerle; „A Tuneable and Highly Parallel Picolitre-Dispenser Based on Di8rect Liquid Displacement "; Sensors & Actuators A 103 (2003); pp. 88 - 92

[9] C. P. Steinert, I. Goutier, O. Gutmann, H. Sandmaier, M. Daub, B. de Heij, R. Zengerle; "A Highly Parallel Picoliter Dispenser with an Integrated, Novel Capillary Channel Structure"; Sensors & Actuators A; available online since 19.05.2004

[10] M. Madou, G. Kellogg; "The LabCD, A centrifuge-based microfluidic platform for Diagnostics", SPIE Proceedings, 1998

[11] G. Ekstrand, C. Holmquist, A. Edman Örlefors, B. Hellman, A. Larsson, P. Andersson; "Microfluidics in a Rotating CD", µTAS 2000, 311-314

[12] M. Madou et al.; "A novel design on a CD disc for 2-point calibration measurement", Sensors and Actuators A 91, 2001

[13] M. Madou, L. J. Lee, S. Daunert, S. Lai, C-H Shih; "Design and Fabrication of CD-like Micro-fluidic Platforms for Diagnostics: Microfluidic Functions", Biomedical Microdevices 3:3, 245-254, 2001

[14] www.bio-disk.com

[15] J. Ducrée, T. Brenner, T. Glatzel, R. Zengerle; „A Coriolis-Based Split-and-Recombine Laminator for Ultrafast Mixing on Rotating Disks"; Proc. µTAS 2003; October 5-9, 2003; Squaw Valley; California; USA; pp. 603 – 606

[16] T. Brenner, T. Glatzel, R. Zengerle, J. Ducrée; „A Flow Switch Based on Coriolis Force "; Proc. µTAS 2003; October 5-9, 2003; Squaw Valley; California; USA; pp. 903 – 906

[17] T. Brenner, Stefan Haeberle, Roland Zengerle, and Jens Ducrée; „Continuous Centrifugal Separation of Whole Blood on a Disk"; MicroTAS 2004, Malmö, Sweden, September 26-30, 2004

[18] O. Gutmann, R. Niekrawietz, R. Kuehlewein, C.P. Steinert, S. Reinbold, B. de Heij, M. Daub, R. Zengerle; "Non-Contact Production of Oligonucleotide Microarrays Using the Highly Parallel TopSpot Nanoliter Dispenser"; to be published in "The Analyst"; 2004

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