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4. Workshop

“Chemische und biologische Mikrolabortechnik”

26.-28. Februar 2008
Ilmenau/Elgersburg (Thüringen)

Programm

Dienstag, 26.02.08

13.00 Uhr Opening

13.10 Opening lecture

A. DeMello: Intelligent synthesis of nanomaterials using microfluidic reactors

T - Microfluidic Systems - Fluidic Transport

13.50 M. Schneider: Acoustic driven flow to mimic blood on a chip

14.30 D. Snita: DC-Electroosmosis in submicrochannels

14.50 M. Pribyl et al.: AC-electroosmosis along systems of coplanar electrodes

15.10 D. Boskovic: Comparative characterisation of static mixing microdevices by residence time distribution measurement and modelling

15.30-15.50 coffee break/Pause

MF - Multiphase-Flow

15.50 M. Kreutzer: Transport phenomena in segmented flows in microchannels

16.30 A.T. Giannitsis: Electroactuation of water droplets

16.50 M. Kielpinski et al.: Selbstkontrolle in fluidischen Netzwerken

17.10 D. Malsch et al.: Droplet-internal flow

17.30 M. Günther et al.: Einfluß von Viskosität, Flußrate und Tensiden auf die Adressierung von Konzentrationsräumen in der Fluidsegmenttechnik

18.50 - 19.20 Postersession

19.30 Dinner

Mittwoch, 27.02.08

C - Lab Micro Reactors

8.30 R. Lammertink: Membranes and microfluidics

9.10 P. Watts: Continuous flow synthesis in microreactors

9.50 H. Löwe: Chemical Micro Processing for Improvement of Chemical Syntheses - From Teaching Students to Advanced Materials

10.10 S. Löbbecke: Use of microreactors for the synthesis of 5-hydroxymethylfurfural (HMF) - a promising chemical building block

10.30-10:50 coffee break/Pause

D - Micro and Nanoparticles in Microfluidic Systems

10:50 C. Sönnichsen: Continuous flow synthesis of noble metal alloy particles

11.30 C. Serra: Synthesis and assembly of size-controlled polymer beads and capsules in an axisymmetric co-flow system

12.10 N. Steinfeldt: Generation of Pt-Nanoparticles in continuous flow operation using microstructured devices

12.30 - 14.00 lunch

E. Combinatorial Chemistry and screening

14.00 K.H. Wiesmüller: Optimization of drug combinations by a cellular diffusion assay

14.40 H. Mathis: AKTIVATES: Mikrozellensysteme für die Wirkstofftestung

15.00 G.A. Gross: Combinatorial Organic Synthesis by Means of Micro Laboratory Techniques

15.20 K. Martin: Modellsystem für die serielle Durchführung tropfenbasierter zellulärer Assays

15:40 A. Schober: Synthesis and Screening Assays

16:00 opportunity for winter evening forest walking (for example: Hohe Warte)

19.00 conference dinner at castle Elgersburg

Donnerstag, 28.02.08

B2 - Microfluidics for biomolecule and cell techniques

8.30 J. Lerchner: Bioreactor monitoring mit Chip-Kalorimetern

9.00 S. Sinzinger: Cell sorting in integrated micro optic fluidic systems

9.20 Ch. Erler et al.: Theoretische und rasterkraftmikroskopische Untersuchungen zur Kraftwirkung elektrischer Wechselfelder auf polarisierbare Partikel

9.40 St. Wiedemeier et al.: Immunisierung mittels Mikrofluidik zur Therapie des Diabetes mellitus

10.00 G. Gastrock et al.: Sterilisierbares Mikroventil mit PNIPAAm-Hydrogelaktuator für Lifescience-Applikationen

10.20 J.T. Schumacher: Module zum simultanen Mischen und Prozessieren von Suspensionen in der Mikrofluidik.

10.40-11.00 coffee break

B3 - Multicellular Systems and toxicological monitoring

11.00 H. Zimmermann: Cryomicrotechnology: enabling technology for vaccine research and regenerative medicine

11.40 A. Funfak: Mikrofluidsegmenttechnik für miniaturisierte toxikologische Untersuchungen

12.00 U. Fröber: Biomikrosysteme zur Kultivierung von Indikatororganismen für Umwelt-Monitoring, Gefahrstoffdetektion und Zytodiagnostik

12.20 J. Schemberg et al.: The pipe-based microreactor

12:40 S. Wölfl: Online monitoring of toxic effects in cellular tissue model systems using BIONAS biosensor chips

13:00 lunch

F - Sensorics, Arrays and Analytical Microsystems

14.10 I. Moser: Bioanalytische Systeme in der Labordiagnostik

15:00 W. Bodemer: Requirements for sensitive detection of biologically relevant macromolecules

15.20 K.-H. Feller: Einsatz von Farbsensoren in der Mikroanalytik

15.40 A. März: On-line quantization of organic analytes by micro flow-through Surface Enhanced Raman Spectroscopy (SERS)

16:00 S. Julich: On-chip PCR: Technology and prospects

16.20 Final Remarks

Intelligent Synthesis of Nanomaterials using Microfluidic Reactors

S.Krishnadasan, J.C. deMello & A.J. deMello

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Nanomaterials exhibit optical and electronic properties that depend on their size and shape, and are seen as tailored precursors for functional materials in biological sensing and optoelectronics. These critical dependencies indicate that 'bottom-up' approaches for nanomaterial synthesis must provide for fine control of the physical dimensions of the final product. Synthetic routes involve the particle growth on an atom-by-atom basis, and have attracted interest owing to their versatility and ease of use. For many applications deviations about the mean particle diameter must be <1% to achieve the desired selectivity. This is beyond the tolerance of standard macroscale syntheses, and it is almost always necessary to use some form of post-treatment to extract the desired particle size. Accordingly, nanoparticles with narrow size distributions can only be extracted, through complex, costly and low-yielding routes. An ideal recipe for nanoparticle synthesis must ensure that nucleation of solute molecules occurs on a timescale that is short compared with the characteristic growth time (in which nuclei capture dissolved solutes). Moreover, nucleation and growth should occur in an environment in which chemical state functions are precisely controlled.

Microfluidic systems provide an ideal medium for nanoparticle production. Since both mass and thermal transfer are rapid, temperatures may be defined with precision or varied on short timescales. Additionally, reagents can be rapidly and efficiently mixed to ensure homogeneous reaction environments, while allowing for additional reagents to be added at predefined times. I will demonstrate how microfluidic reactors can be used to perform highly efficient nanomaterial synthesis and also report recent studies on 'intelligent' synthesis of nanoparticles of varying size, shape and size-distribution.

T
Microfluidic Systems – Fluidic
Transport

Acoustic Driven Microfluidics to Mimic Hydrodynamics Forces in our Veins

Matthias F. Schneider
University of Augsburg, Germany

Miniaturization has always been one of the main goals of modern technology. Reducing, for example, the amount of substance for a medical or biochemical assay, not only represents a major reduction in price, but can even open new fields of research and provide fundamental scientific insights. One of the fastest developing fields in this context is microfluidics, in which scientists and engineers face the problem of mixing and moving small amounts of liquids at low Reynolds numbers. Nature came up with flagella and ciliae to propel bacteria around in the pond but researchers all over the world have to continue to look for new technologies to mix and pump small amount of liquids.

In this talk I will present a new technology, which allows the control of microfluidic systems down to the sub-picoliter range using Surface Acoustic Waves (SAW) as a microfluidic pump. I will present how this technology can be applied in both i) applied and ii) basic science.

i) As a tool as it may be used in biotechnology we use our lab-on-a-chip system to study the dynamics of mixing under laminar flow conditions. Furthermore we show how to control transport of complex liquids (emulsions, water, oil and blood) as well as dispensing them in small packages. Integrating heaters and capacitors into the planar technology allow the performance of PCR directly on the chip. We also study the physics of cell adhesion and the uptake of nanoparticles (both for drug delivery and health risks) under hydrodynamic conditions and for medical diagnostic purposes.

ii) Using multiple acoustic pumps and modern surface functionalization simultaneously enabled us to study fundamental problems in fluid mechanics such as the dynamics of eddies and their interaction with particles in flow and the problem of chaotic advection in 2D and 3D. Furthermore, we accomplished an in vitro model for the investigation of the dynamics of the initial steps of blood clotting on a single molecule level. We demonstrate for the first time , that this initial step of blood clotting is a self organized process.

DC electroosmosis in micro- and submicrochannels

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Key words: DC electroosmosis, ionexchange membrane, microfluidic systems, mathematical modeling

Spatially two-dimensional non-equilibrium mathematical model based on the coupled mass balances, Poisson, Navier-Stokes and Nernst-Planck equations describing electroosmotic flow through a sub-micron channel with a constant electric charge fixed on the channel walls is presented. This system is governed by the hydrodynamic, electrostatic and mass transport phenomena. The non-slip boundary conditions are employed. The effect of an imposed electric field on the system behavior is studied by means of a numerical analysis of the model equations. Physically relevant sets of the model parameters are used. We have obtained the following findings. If the channel width is comparable to the electric double layer dimension, the system behaves as an ion-exchange membrane and the dependence of the electric current passing through the channel on the applied voltage is strongly nonlinear. In the case of negatively (positively) charged walls, the narrow region of very low conductivity (ionic gate) is formed in the free electrolyte near the channel entry facing the anode (cathode) side. For a wide channel, electric current is proportional to the applied voltage and the velocity of electrokinetic flow is linearly proportional to the electric field strength. Complex hydrodynamics, e.g., eddies formation, and existence of ionic gates, is the most interesting characteristics of the studied system. Hence, current-voltage curves and relevant spatial distributions of the model variables at selected points are studied and described in detail.

AC electroosmosis along systems of co-planar electrodes

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Key words: AC electroosmosis, interdigitated electrodes, microfluidic systems, mathematical modeling

The results of mathematical modeling of electrokinetic flow forced by AC electric fields are reported. Electrokinetic pumps are widely used in microfluidic and nanofluidic devices for the electrolyte transportation. The electroosmotic transport can be induced in a structure containing an electrolyte and an electric charge bound on a liquid-solid interface. Contrary to the classical DC pumps, the electrochemical reactions need not take place in the AC pumps due to low voltage amplitude (typically less than one volt). In principle, there are two co-planar arrangements of AC pumps that are exploited in microfluidic devices: (i) array of asymmetric co-planar interdigitated electrodes, (ii) array of co-planar symmetric electrodes. In the former arrangement, the same AC electric field is imposed on each pair of the interdigitated electrodes. The latter arrangement relies on a phase shift that is applied on neighbouring electrodes.

Geometric (the electrode size, the distance among the electrodes, and the depth of the microchannel), electrolyte (concentration and the pH value) and electric field properties (amplitude, frequency, and phase shift) of a microfluidic system have to be found in order to maximize the flow rate. Thus the parametrical space is huge and multidimensional. To accelerate the development of experimental AC micropumps, we have derived and analyzed a non-equilibrium mathematical model of the AC electrokinetic flow above systems of co-planar electrodes. The model is based on mass, momentum, and molar balances and the Gauss's law of electrostatics. The non-slip boundary conditions are considered.

We investigate three particular co-planar systems in this work: one asymmetric and two symmetric in three- and four-phase arrangements. Dependences of flow rate on frequency, amplitude and other parameters are obtained and discussed. The development of stable periodic regimes from a steady state (immovable electrolyte) is analyzed and duration of the transient is evaluated. Examples of spatio-temporal fields of electrolyte concentration, electric potential, pressure and velocity are also presented.

Comparative characterisation of static mixing microdevices by residence time distribution measurement and modelling

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The analysis of the overall flow behaviour by means of residence time distribution (RTD) theory provides essential information of a chemical reactor performance. Even though RTD measurements are often based on a black-box consideration, basic flow characteristics of reactors can be derived without applying complex modelling and simulation techniques.

Particularly in microfluidic devices a wide range of different RTD characteristics can be achieved depending on the channel dimensions, flow rates and efficiency of incorporated passive mixing structures.

Within a Re number range of two orders of magnitude the integral flow characteristics can change considerably. This can clearly be investigated by RTD measurements.

In this work, an input-response technique for the measurement of the residence time distribution of microfluidic static mixers was developed. The measurement setup ensures interchangeability of microfluidic devices and thus allows characterisation and comparison of different devices comprising different microchannel geometries and mixing structures.

The evaluation of the raw data obtained by the experimental procedure requires a model description of the RTD for each considered microfluidic device. For the whole range of Re numbers in which microdevices can be utilized it is usually not possible to apply the same model, even if the same device is used. Various theoretical and empirical models were applied and the advantages and disadvantages of the different types of models were investigated.

The analysis of the RTD of a microfluidic mixer at various flow rates indicates in most cases a transition from diffusion controlled mass transport to faster and more effective convective mass transport. Consequently, it is possible to define an optimal operational area for a certain microdevice particularly with regard to more effective use. Comparison of different devices enables more efficient selection of microstructures for a certain application.

MF

Multiphase-Flow

Transport Phenomena in Segmented Flows in Microchannels

Michiel Kreutzer, Chris Kleijn, Volkert van Steijn, Freek Kapteijn,
Jasper Bakker

Fluid interfaces provide unique opportunities for microfluidic and nanofluidic systems. Applications range from microscale heat exchangers and miniature fuel cells to microreactors for materials synthesis. Multiphase flow in such devices can be challenging, as the interfacial forces naturally favor axisymmetric geometries that are difficult to microfabricate. The advantages of surface tension dominated microfluidics include a much richer dynamic flow behavior and enhancement of heat and mass transfer by creating secondary flows. These advantages offer many uses beyond enabling gas-liquid and fluid-solid reactions.

In particular, we are interested in segmented flow of gas and liquid in hydrophilic channels. This flow pattern has several key features for reaction and assay purposes. The presence of bubbles reduces the amount of dispersion of liquid flowing through the channels, ensuring that reactants and products spend a uniform amount of time in the system. For particle synthesis in microfluidic systems, a uniform residence time distribution translates into narrowly distributed particle sizes. Liquid segments are efficiently mixed by circulation motion and gas bubbles are separated from microchannel walls by only a thin film (thickness $< 1\mu\text{m}$). Thin films reduce mass transfer resistance to components immobilized on the walls, such as catalysts or analytical reagents and antibodies.

Electroactuation of water droplets

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Living cells can be cultivated in micro-bioreactors which are as small as a water droplet [1]. The development of microfluidic processors, devices that can not only measure behaviour of such droplets but also sort them, is at the leading edge of the today's bioprocessing technology. The intention is to automate time consuming and costly labour using automatic and precise devices that measure and handle bioreactors. Various actuation methods have been proposed [2,3], the most efficient of which – in terms of speed and accuracy - uses electrowetting [4-8].

Electrowetting is an electrokinetic means of manipulating a droplet by electrical modification of its surface tension. The electrokinetic force pulls the droplet along the gradient of the surface energy. A simplified consideration of the electrowetting force assumes that the movement on coplanar electrodes is caused by asymmetric electrowetting, resulting in contact angle hysteresis between opposite sides of the droplet [9]. The difference in the surface tension on both sides causes a displacement force. This force is proportional to the difference of the cosines of the two contact angles. The cosines of the contact angle are given by the Lippmann's equation [10], which extends the well-known Young's equation by a term accounting for the external applied potential.

In our experiments, we investigate the feasibility of using co-planar electrodes coated with an isolating film and a hydrophobic fluoropolymer film atop. The electrified isolating film forms a capacitor that attracts spare ions from the bulk of the drop. These ions modify the energy of water-air and water-substrate interface, which results in the minimization of the surface tension that finally moves the drop. Another reason for adding isolation is to prevent current passing between the drop and the electrodes that results to hydrolysis, heating and deformation. The fluoropolymer film provides sufficient repulsion for the droplet to retain a spherical shape and slide as an entity. We polished the electrodes before coating, to ensure minimal roughness that eliminates the resistive forces. The droplet motion was found to be highly dependent on the applied voltage. The motion is shown in the following Fig. 1.

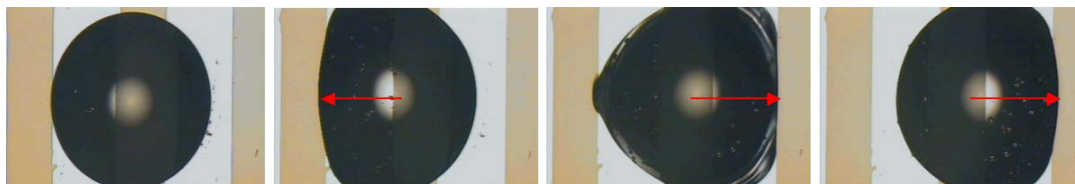


Figure 1 Vibrational motion of a water droplet between two adjacent electrodes. The transition is actuated using a low frequency alternating voltage of 150 Vp. The entire electrode structure is coated with 100 nm Si_3N_4 layer and 100 nm fluoropolymer layer atop. The platinum electrodes were fabricated on a Pyrex glass wafer using standard lithography. The electrodes are 0.5 mm wide and the diameter of the drop ~1 mm.

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Acknowledgements are due to Dr. S. Howitz from GeSiM GmbH, Dresden, Germany, and Dr. K. Schröder from Leibniz Institute for Plasma Research and Technology, Greifswald, Germany, for coating the electrodes and technical discussions.

Application of Self-Control in Droplet Based Microfluidics

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Droplet-based microfluidics provide a powerful platform for high-throughput microchemical operations, applied in micro analytics, micro reaction technology and live sciences. Today's research interests focus on development of highly integrated fluidic networks for sample processing according to a microchemical or microanalytical protocol. Normally, fluidic networks with integrated fluidic loops and bypasses are very complicated systems, that require a huge effort for external control and integration of actor components. In contrast, in droplet based microfluidics interface generated forces can be used, to temporarily seal bypasses or to generate well defined pressure gradients at strictures. This potential can be used, to implement self-control and self synchronization at functional nodes in order to minimize the effort for external control and actors integration. First implementations of this concept have been reported. A device for generation of droplet sequences with alternating composition was reported by the Ismagilov group[1], an element for regulation of droplet traffic at a junction was reported by Cristobal[2]. Devices for controlled droplet fusion[3], or structures for flow synchronization of two droplets in different microchannels[4] have been published. Here we report on development of functional nodes for self synchronized 1:1 coalescence of two independently generated droplet sequences at a Y-shaped junction[5,6] and on approaches for droplet aliquotation at a Y-shaped bifurcation. The droplet-connector automatically balances the time delay between two droplets, arriving at the junction. Therefore, strictures are integrated into the Y-junction and an additional bypass connects the arriving channels. The first arriving droplet stops at the stricture, until its fusion partner arrives. The droplet splitter performs an 1:1 aliquotation of all elements of a droplet sequence. The main challenges are the balancing of pressure differences at the outlets and the correct aliquotation for droplets independent on their volume at a wide range of flow rates. The splitter design is based on the rule, that forces, required for splitting are always lower than the forces, required for complete droplet inflow into only one of the outlet channels without splitting.

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Droplet internal flow in all-glass micro channels

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Segmented flow in all-glass micro channels is characterized by an interplay of interface interaction and phase internal flow field formation. As a result, droplet generation, pressure drop, local shear rates and mixing performance are determined by this interplay. Yet, these effects are strongly influenced by the properties of the employed fluids, the wetting properties of the channel surface and experimental conditions like flow rate and fluid phase ratio. We utilize methods to measure and visualize these effects and show their dependency on fluid properties and experimental conditions.

All-glass micro channels have been prepared by wet etching of glass half channels and anodic bonding of two glass substrates. The channel surfaces are treated with Octadecyltrichlorosilane in order to generate optimum wettability for separation fluid (tetradecane) and minimum wettability for droplets (aqueous). We analyze droplet sequences that completely seal the channel. Direct contact between the aqueous droplet and the channel wall is prevented by a permanent thin film of the separation fluid tetradecane.

Internal flow fields of droplets translating through micro channels are visualized by video sequences and measured by micro particle imaging velocimetry (μ PIV). Phase internal flow has been investigated for the contribution of interface friction to the formation of the internal flow field. Dependent on wetting conditions, the decisive contribution of liquid/wall or liquid/liquid interface friction switches. This results in alternating direction of phase internal flow between micro droplets and separation fluid. For internal flow field evaluation, algorithms of PIV image analysis have been extended for algorithms of droplet recognition, mapping and transformation into a single coordinate system before displacement analysis.[1]

Pressure drop of two-phase flow in micro channels has two major contributions. One is determined by the Laplace pressure difference over the droplet, the second originates from viscous losses in the flow field.[2] We present recent measurements of the pressure drop dependency on fluidic properties and experimental conditions for fluid/fluid two phase flow in all-glass micro channels.

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Einfluß von Viskosität, Flußrate und Tensiden auf die Adressierung von Konzentrationsräumen in der Fluidsegmenttechnik

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Zur Untersuchung der Einflußfaktoren auf die Konzentrationseinstellung in Mikrofluidsegmenten wurden Segmentbildungsexperimente durchgeführt. Diese erfolgten in vom IPHT Jena entwickelten Doppelinjektorchips mit 2×2 -Fluideingängen mit in-situ Mischung im Hauptkanal. Zur Adressierung der zweidimensionalen Konzentrationsräume wurden zwei Farbstofflösungen (Kristallviolett **KV** und OrangeG **OG**) an verschiedenen Injektoren mit Wasser vermischt und so Segmente erzeugt, die unterschiedliche Konzentrationen an beiden Farbstoffen enthielten.

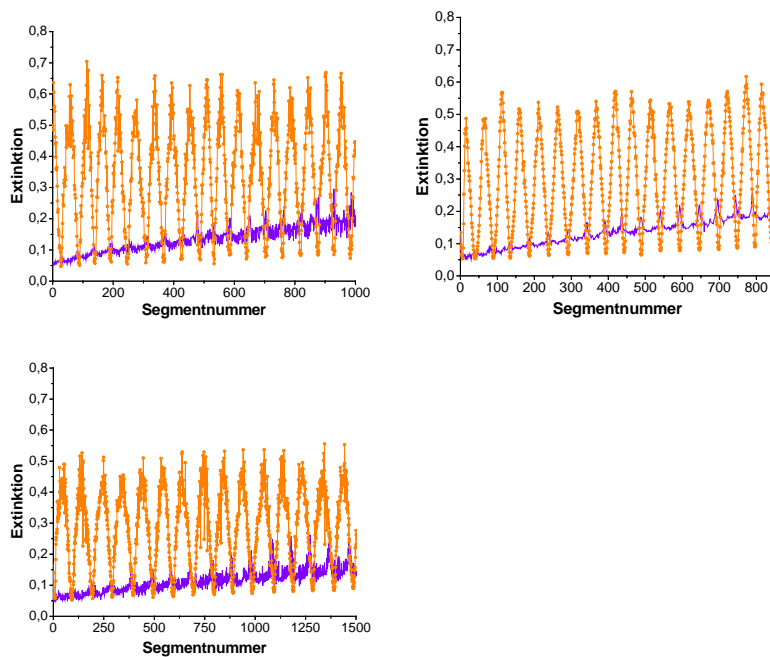


Abb. 1: Erzeugung von Konzentrationsprofilen ohne Glycerinzusatz mit 40 $\mu\text{l}/\text{min}$ Trägerstrom (links), mit 10 % Glycerin in der Farbstofflösung und 40 $\mu\text{l}/\text{min}$ Trägerstrom (Mitte) sowie mit 10 % Glycerin in der Farbstofflösung und 80 $\mu\text{l}/\text{min}$ Trägerstrom; Die Farbstofflösungen aus KV und OG wurden mit Gesamtförderraten von je 20 $\mu\text{l}/\text{min}$ gefördert.

Die Bestimmung der Konzentration erfolgte mittels paralleler Transmissionmessungen bei 480 nm (OG) bzw. 590 nm (KV) und einer Meßfrequenz von 500 Hz, was etwa 200

Messungen pro Segment entsprach. Es wurde festgestellt, daß die Segmentbildung bzw. die Erzeugung von Konzentrationsprofilen signifikant von der Fließgeschwindigkeit des Trägerstromes und der Viskosität der Lösung abhängt (Abbildung 1). Die Zugabe von 10 % Glycerin zu jeweils einer Farbstofflösung verringerte deutlich die Oszillationen der Konzentrationswerte in den Segmenten. Den gleichen Effekt konnten wir beobachten, wenn im verwendeten Aufbau Trägerflußraten von 20 oder 40 $\mu\text{l}/\text{min}$, anstelle von 80 $\mu\text{l}/\text{min}$ verwendet wurden.

Wir danken F. Möller und Stefan Wötzel für die geleistete Unterstützung bei den Messungen zur Segmentbildung und –charakterisierung, S. Schneider für die Softwareprogrammierung und Hilfe zum Aufbau des Versuchsplatzes und Th. Henkel vom IPHT Jena für die Bereitstellung der Doppelinjektorchips sowie dem BMBF (FKZ 16 SV 1999) für die finanzielle Unterstützung.

C
Lab Micro Reactors

Membranes and microfluidics

Rob G. H. Lammertink

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Porous media find extensive use in chemical and pharmaceutical industry. However, their use in microreactor and microfluidic devices is still limited. Here, the concept for exploiting porous layers (i.e. membranes) within microstructured reactors is studied. A porous structure can be utilized for several functions, including gas-liquid contacting, separation, and dosing. For gas-liquid contacting, the porous layer needs to be non-wetting for the liquid phase in order to reduce mass transport limitations. During contacting, the gas phase can diffuse easily through the porous matrix, while the liquid is contained inside the microfluidic channels.

The approach for gas-liquid contacting in microfluidic channels is demonstrated by detecting simple carbon dioxide gas dissolution. By measuring the conductivity of the solution dynamically, mass transport kinetics can be obtained. The experimental results were compared to numerical simulations.

This concept is demonstrated for both polymeric as well as ceramic microreactors. Ceramic microreactors can be fabricated by a micromolding method followed by a polymer binder burnout and sinter treatment. The resulting alumina structures are open porous with sub micrometer pore sizes. They can be easily modified by using silane chemistry to obtain the desired surface properties.

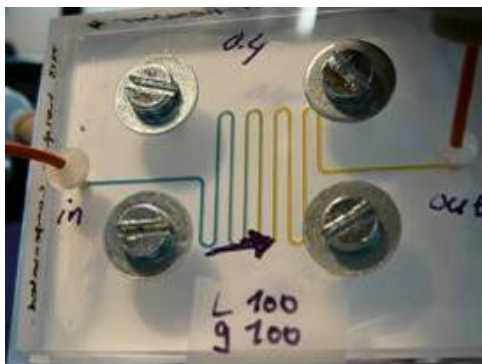


Fig. 1

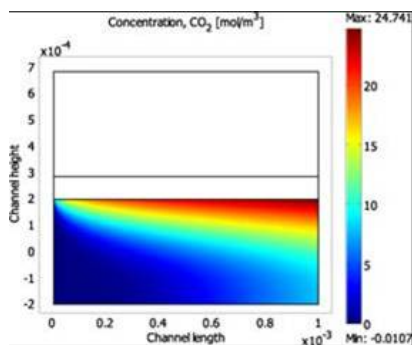


Fig. 2

Continuous Flow Synthesis in Micro Reactors

Paul Watts

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The miniaturisation of chemical reactors offers many fundamental and practical advantages of relevance to the chemical industry, who are constantly searching for controllable, information rich, high throughput, environmentally friendly methods of producing products with a high degree of chemical selectivity.

In this presentation a number of chemical reactions of industrial interest will be used to illustrate the advantages that micro reactors offer for the rapid optimisation of reactions, in which the product is typically produced in both higher yield and purity. It will be illustrated that compounds may be prepared and purified within an integrated system and that it is possible to generate intermediates *in situ* within the reactor, which may then be subsequently reacted to produce more complex products. More recently the incorporation of solid supported reagents and catalysts has been investigated and the results will be discussed. The use of solid supported reagents adds even greater diversity to the range of reactions that may be achieved within such systems. It will be demonstrated that the dimensions of reactors may be increased in size while maintaining the classic advantages associated with miniaturisation. In such systems significant quantities of analytically pure compound may be prepared without additional purification.

It will be illustrated that the 'scale out' of micro reactors may be used to generate larger quantities of compound without having to re-optimize the reaction, thus saving time and money whilst simultaneously making the process inherently safe. Furthermore, integration of the microfluidic system with real-time analytical detection will be illustrated enabling *in situ* process control to be achieved.

Chemical Micro Processing for Improvement of Chemical Syntheses - From Teaching Students to Advanced Materials

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The use of microreactors, or more exactly described as microstructured reactors in the context of the process-oriented contents presented here, is without any doubt an accepted method in chemical research as well as in some cases in industrial applications. In the recent years many papers were published to describe the microfabrication of microstructured reactors, especially for the fabrication of microchannels, and examples for the their application in chemical processing were given. Meanwhile it becomes a common experience underlined by theoretical evidence, that the internal size of such channels has not to be set necessarily as small as possible by today's state-art-of-the art for manufacturing but the size depends on the demands of the chemical reaction itself.

Thinking consequently in this direction means a paradigm change in chemical processing. It is no longer necessary to adjust the protocol to perform a chemical reaction to a given equipment. Contrarily, the chemical reaction can now be driven close to the kinetic limits due to the superior mass- and heat transfer properties of special designed microstructured reactors. If this prediction is true, the chemical protocol has to be changed dramatically, i.e. to a continuous flow system, high reaction temperature, high pressure or otherwise unusual process regimes. Consequently a "novel" chemistry arises.

In view of these advantages in chemical processing skilled personal is vital to transform common chemical protocols to an applicable chemical micro processing procedure. Therefore, students of chemistry and chemical engineering should get fundamental knowledge of chemical micro processing, the possibilities, advantages and drawbacks of this comparably new technology.

Some examples will show how teaching and education of chemical micro process technology can be combined with the experimental use of microstructured reactors:

- Microwave assisted Microreactor Processing (μ^2): Synthesis of Ionic Liquids,
- Synthesis of biphenyl by high-temperature pyrolysis of benzene,
- Hydrogenation of nitrobenzene on Pd-covered nickel foam,
- Synthesis of inorganic/organic block polymers with temperature responsive properties.

To give a brief overview, the first two examples will be explained here.

Although a simple chemical reaction at first sight, the synthesis of ionic liquids suffers from the huge amount of heat released during the reaction. For example, an equimolar batch reaction of 1-methylimidazole and hexylbromide (0.0625 mol each) gives an

immediate temperature rise from 0°C to 185°C, when mixed all at once. To perform such an experiment can thus be very dangerous. The students textbook suggest only one way out, namely to add the alkylating agent dropwise under reflux and heavy stirring. Examining the opportunity to prepare the ionic liquid 1-methyl-3-hexyl-imidazolium-bromide in a continuous way the mixing of the reactants was performed in a micromixer and reaction was supported by subsequent micro-wave irradiation. Experiments were performed in a PARR Multiwave 3000TM microwave oven equipped with a ducts for insertion of tube to ensure a continuous flow. As reactor a micromixer-tube setup made of PEEK and PTFE was used. The tube diameter was 1/8" and different residence times could be adjusted by the tube length as well as by the flow rates of the supply pumps. As a result it can be turned out that a low microwave energy-supply, partially less than 50 W, and a short residence time of approx. 10 min led to a complete conversion of the imidazole. At higher microwave energies (>200W) the residence time could be shortened to less than two minutes, demonstrating the impact of alternate energy sources on process intensification.

The feasibility of microstructured reactor processing for high-temperature pyrolysis of benzene to biphenyl at 800°C was the aim of the second experiment. Such kind of (non-functional group assisted) reaction plays normally no role in university education. Nevertheless, it is a good example how to perform organic reactions under very unusual conditions. Pyrolysis reactions are mostly of radical nature and therefore very fast but with low selectivity. The main products for this reaction are low molecular hydrocarbons and coke while the formation of biphenyl is not preferred. To increase the yield of biphenyl the residence time should be short and fast heating and quenching of the reaction mixture is necessary. Pure benzene is evaporated and overheated in microstructured systems and the pyrolysis itself, i.e. the transformation of benzene into biphenyl, is also performed in a special designed microstructured reactor. The used of microstructured devices in the laboratory set-up should ensure an extremely fast heating of benzene from room temperature up to 850°C within milliseconds. High yields of 30% of biphenyl, compared to the respective data given in literature with approx. 1-3%, could be achieved. Under pyrolytic conditions a heavy coke formation takes place even at short residence times below 500 ms, and the high yields decrease rapidly with the increase of time on stream of the reactor. Consequently, the high-temperature pyrolysis of benzene to biphenyl is a good example to show possible drawbacks of a microstructured reactor and to make clear that chemical micro processing is a useful but not almighty tool.

Use of microreactors for the synthesis of 5-Hydroxymethylfurfural (HMF) – A promising chemical building block derived from renewable resources

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The interest in renewable resources as chemical feedstocks is growing considerably fast. For example, furan derivatives obtained from renewable carbohydrates such as fructose have a huge potential to become sustainable substitutes for petroleum-derived chemical components. In particular, 5-hydroxymethylfurfural (5-HMF) is considered as a key building block for the synthesis of 2,5-furandicarboxylic acid, furandiamine and other derivatives used for polymer synthesis [1-5].

Today, the synthesis of 5-HMF from renewable resources is usually based on the catalytic dehydration of mono- and polysaccharides using organic acids (e.g. oxalic acid), inorganic acids (e.g. H_3PO_4 , HCl), zeolites or inorganic salts (e.g. $MgCl_2$). However, all those batch processes show considerable limitations in performance. When dehydration is conducted in pure water at high temperatures only low selectivities and a wide variety of by-products are obtained. On the other hand, processing at low temperature give certainly fair selectivities (>70%) but only poor conversion of < 50% can be achieved. To overcome restrictions in conversion and selectivity dehydration processes have been conducted in biphasic systems based on water and an organic co-solvent such as methyl-isobutyl-ketone (MIBK), 2-butanol or dimethylsulfoxide (DMSO) [1-2], but also in sub- and supercritical mixtures of acetone/water, in sub- and supercritical methanol as well as in subcritical acetic acid [4-5]. Although conversion and HMF selectivity could be improved in comparison to conventional HMF syntheses the major drawback of these processes is their negative impact on the sustainability of the process since organic solvents are used that are partly hazardous, harmful to the environment and raise process costs significantly.

In recent years continuously operated microfluidic reactors have received an increasing interest since they allow applying new process windows to chemical reactions as a result of improved mass and heat transport characteristics. Here, we report on the microreactor-based green synthesis of 5-hydroxymethylfurfural in water avoiding the use of any organic co-solvent. The process conditions were deliberately shifted towards elevated temperature and pressure regimes (up to 180°C and 20 bar) in combination with short residence times (1 - 3 min) to significantly increase space/time yields and achieve satisfying selectivities of >75%.

Financial support by the Deutsche Bundesstiftung Umwelt (German Environmental Foundation) is gratefully acknowledged.

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D
Micro and Nanoparticles in
Microfluidic Systems

Continuous Flow Synthesis of AuCu Nanorods

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The physical and chemical properties of nano-sized materials rely strongly on their crystal and surface structure as well as on their material constitution. We present a continuous flow synthesis of gold and gold copper nanorods allowing tailored design of particles with desired shapes and online monitoring of particle growth. Gold and gold copper nanorods have been synthesized using the seed-mediated approach. The influence of different ratios of metal salts in the growth solutions on the growth and shape of the nanorods was investigated by optical spectroscopy and electron microscopy techniques, respectively. It has been found that the growth and the optical properties are strongly influenced by the presence of copper. Additionally, the optical properties of the particles have been characterized by single particle spectroscopy using a dark-field microscopy setup.

Synthesis and assembly of size-controlled polymer beads and capsules in an axisymmetric co-flow microsystem.

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We report on the use of an axisymmetric microsystem for the synthesis of monodisperse polymer particles ($CV < 5\%$), either beads or capsules, in the size range of tens to several hundred of microns. The emulsification of the monomer phase is achieved in a very simple capillary/tubing co-flow microsystem without any surfactant or surface treatment. Polymer beads are obtained after UV irradiation while polymer capsules are generated within the microsystem through an interfacial polycondensation reaction. A preliminary study has shown that in the dripping regime the variation of particle diameter with respect to operating parameters (flow rates and viscosities of the dispersed and continuous phases) is fairly well described by an empirical relationship between two dimensionless numbers, the normalized particle diameter and the Capillary numbers ratio. This co-flow microsystem also allows for the preparation of inorganic-organic hybrid beads as well as hollow and doped capsules. By confining the particles in the microsystem, we successfully prepare polymer beads necklaces with different arrangements (zig-zag, linear).

Generation of Pt-Nanoparticles in continuous flow operation using microstructured devices

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Small metal particles in the nanometer range display chemical and physical properties which differ from bulk materials. Therefore, nanoparticles play an important role in catalysis both in homogeneous and heterogeneous phases [1]. A well-known process for synthesis of precious metallic nanoparticle is the polyol method. Here a metal salt is reduced in the presence of a stabilizing agent that prevents the nanoparticles from agglomeration. Essential factors that control the particle size are the strength of the metal-metal bond, the molar ratio of metal salt/colloidal stabilizer/reducing agent, the extent of conversion or the reaction time as well as the applied temperature and pressure [2]. This great number of factors makes it very time consuming and difficult to study the influence of these parameters on particle shape, size and particle size distribution in a batch reactor.

Microstructured reactors offer the possibility for preparation of nanoparticles under controlled conditions in a continuous process [3,4]. Beside the increasing speed of studying the influence of reaction conditions, microstructured reactors offer further advantages. Using microreactors convective mixing and interdiffusion can be controlled better than in a batch reactor. The narrow channel dimensions combined with micromixers provide millisecond mixing times. The small channel size prevents hot spots typically generated in batch reactors and enable an exact adjustment of temperature along the microchannel. Therefore, nucleation can take place in a homogeneous chemical environment which is important for achieving monodisperse nanoparticles. The continuous flow regime also allows the addition of further reagents downstream directly after nucleation.

The polyol synthesis method [5] was applied for the preparation of Pt-nanoparticles using microstructured devices. First experiments were carried out in a micro tube reactor made of glass using the same mixture than for batch experiments. Here temperature, residence time and the position of stabilizing agent addition (poly vinylpyrrolidone) were varied. The formed Pt-nanoparticles are characterized by UV/Vis, small angle X-ray scattering (SAXS) and transmission electron microscopy (TEM) and compared with those obtained from the batch process. For further experiments a micromixer combined with a residence time modul is applied. In these experiments the influence of Pt-salt and PVP concentration on particle size and size distribution is studied using the above mentioned characterization methods. Results of catalytic tests of supported Pt-nanoparticles are also presented and discussed.

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E
Combinatorial Chemistry and
Screening

Optimization of drug combinations by a cellular diffusion assay

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For an improved treatment of cancer the identification of optimized drug combinations for individual patients is a promising approach. A major requirement for such investigations is the rapid testing of a moderate number of different compound combinations with a limited amount of tumour cells.

We developed a device for the combinatorial generation of compound mixtures by diffusion with concentrations over several orders of magnitude. Diffusion takes place in cell compatible polymer matrices such as agarose or gelatine. Mixtures of test compounds are generated by a superposition of concentration gradients (2D-gradient). Only two pipetting steps are required to generate a series of assays that only depends on the spatial resolution by which the diffusion gradient is converted into a biological assay. The diffusion device is compatible with 12-well microtiter plates enabling the efficient testing of combinations of different compounds. Data are generated by automated image acquisition and image analysis. The device is applicable for all diffusible compounds such as small molecules, peptides, lipopeptides and antibodies.

Here we present the investigation of anticancer drug combinations against different leukemia cell lines. The induction of apoptosis is measured by annexin staining using fluorescence microscopy.

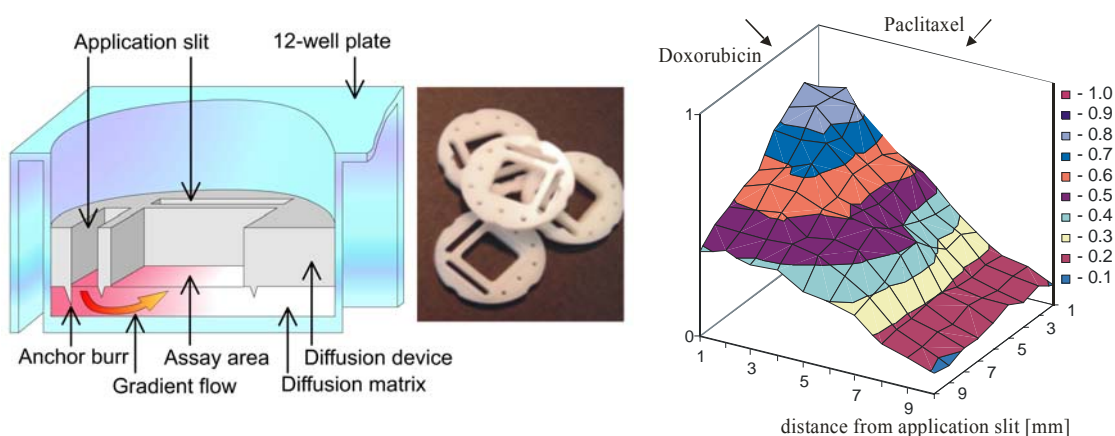


Figure 1: a) Scheme and photograph of the diffusion device placed on an agarose patch in a well of a 12-well plate. Cells embedded in a collagen matrix are poured into the central opening. b) Induction of apoptosis by combination of Doxorubicin and Paclitaxel.

Doxorubicin was applied at a concentration of 345 μM , Paclitaxel at a concentration of 50 μM . Jurkat cells were exposed to the substances during gradient formation for 16 h. Death of Jurkat cells, stained with the cell tracer CFSE, is expressed as the ratio of annexin over CFSE signal.

AKTIVATES: Mikrozellsysteme für die Wirkstofftestung

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In ethischer wie wirtschaftlicher Hinsicht sind Tierversuche stark ins Kreuzfeuer der Kritik geraten. Die Aussagekraft von sogenannten „Tiermodellen“ ist außerdem sehr beschränkt, wie vor allem an den jüngsten Misserfolgen mit rekombinanten Proteinpräparaten erkennbar wurde.

Die EU-Kommission bringt die REACH-Verordnung zur Umsetzung, nach der die Testung jeder Substanz mit einer gewissen Jahrestonnage vorgeschrieben ist. Gleichzeitig -und das scheint zunächst ein Widerspruch zu sein- soll die Anzahl der Tierversuche drastisch reduziert werden. Das österreichische Umweltbundesamt (siehe www.ubavie.gv.at) geht davon aus, dass 70% der benötigten Tierversuche für die REACH-Verordnung durch alternative Methoden ersetzt werden könnten. Ähnliche Überlegungen werden vom deutschen BMBF und deutschen Umweltbundesamt angestellt.

Im Bereich der Medikamententestung reduzieren aussagekräftige Methoden die Zeit für Zulassungsverfahren deutlich und damit die Kosten drastisch.

AKTIVATES setzt bei beiden Applikationen mit der Entwicklung eines Mikrosystem-basierten Ansatzes an: verschiedenen Zelllinien ausdifferenzierter embryonaler Mausstammzellen werden in Mikrokompartimenten unter idealen Bedingungen kultiviert und schließlich einer Testung mit Wirkstoffen oder chemischen Verbindungen zugänglich gemacht.

Die Datenaufnahme erfolgt elektrisch (elektrophysiologisch) und optisch, die Informationsverarbeitung nachfolgend algorithmisch. Ziel des Projektes ist die Entwicklung eines Komplettsystems (BioMST).

Das System ist in eine Sensorik und eine Readereinheit für die Datenaufnahme eingebunden.

Das Anfluten der Wirksubstanzen erfolgt mithilfe einer Fluidik.

Wir präsentieren Konzeption und aktuellen Stand des laufenden Projektes und zeigen Ergebnisse aus den Vorprojekten.

Combinatorial Organic Synthesis by Means of Micro Laboratory Techniques

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Abstract

Micro laboratory techniques enable improved experimental output for synthesis and screening applications. The combination of experiment miniaturization and process automation merge the advantageous of minimal material consumption with high sample number processing.

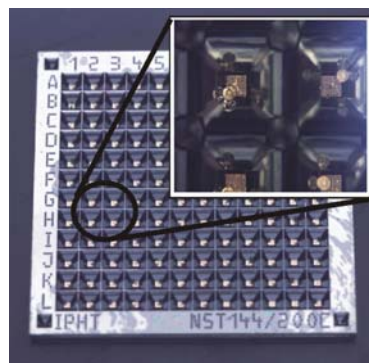
In the field of organic synthesis as well as material research combinatorial synthesis techniques are well established. Synthesis optimization as well as combinatorial probe sampling is used intensively in both fields. For drug discovery research the generation of information about the structure / effect relation is the predominant aim. Only a very low amount of substance is necessary for the determination of appropriate parameters. Usually the necessary amount is much lower than the synthesized once because the classical synthesis techniques were developed with respect for a safe and comfortable handling by hands. Here, automated micro laboratory techniques can help to increase the sample throughput and decrease the material consumption for one data point.

Various complementary approaches for miniaturized synthesis were realized by means of micro system technologies. Therefore, solid supported synthesis methodology as well as solution phase synthesis were combined with different micro system technologies. Different complementary approaches for miniaturized synthesis were developed. Each method offers specific benefits for specific synthesis applications. But, each method demands for the integration in existing laboratory processes specific interfaces.

Following four micro laboratory techniques for the combinatorial synthesis are discussed:

Specially encodes single bead synthesis in 100 nl reaction scale

Micro structured arrays of reaction vessel were fabricated in silicon and filled individually with single solid phase beads. Each reaction vessel was equipped with a micro sieve at the bottom which enabled the removal of reaction solution by applying vacuum under the array. Liquid- and bead handling techniques were developed as well as an appropriate array periphery which enables reaction temperatures up to 120° C. A library of 100 compounds was prepared to prove the principle. current method is well suited for the highly parallel synthesis of large compound libraries at nmol scale. [1, 2]



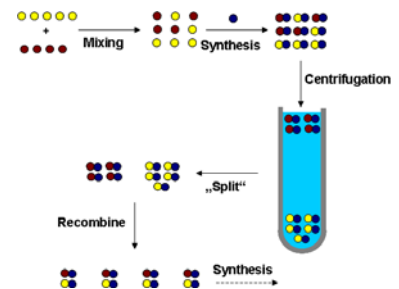
Directed sorting synthesis using solid phase synthesis chip and a magnetic clip board technique

Glass chips bearing a magnetic core were coated with a polymeric PEGA film suited for solid supported synthesis. 60 chips were arranged on a magnetic carrier clipboard made of glass. The magnetic forces were optimized to keep the chips attached even if streaming forces exist under reaction conditions. A robotic system equipped with a gripper was assembled to realize automated directed sorting. Single chips were rearranged between different carriers subsequently to synthetic building block immobilization step. The so called Syn&Sort system was used exemplary for the preparation of a peptide library.[3] Current method is well suited for the preparation of libraries with up to 1000 members at low μmol scale.



Split and recombine combinatorial synthesis principle using a density based fraction separation principle

Polymeric beads with different densities can be prepared by embedding inert filling materials. Suspension polymerization, in presence of SiO_2 particles as filling material, was used to prepare beads with different densities. Mixtures of these beads with different densities can be used for a split and recombine synthesis and separated by their density using a sedimentation principle. The principle seems to be suited for huge libraries at nmol scale.



Sub μl solution phase synthesis using the serial micro segmented flow principle

The segmented flow principle enables the serial processing of sub- μl samples in a serial manner. If appropriate separation liquids and solvents are used solution phase reactions can be done even at high temperature. The superior advantage of the segment flow principle is the serial processing possibilities which enable the handling of huge probe numbers. Exemplary results for the combinatorial synthesis in micro segments as well as polymeric material research will be given [4, 5]. Current method is well suited for solution phase library synthesis in a μmol range.



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Modellsystem für die serielle Durchführung tropfenbasierter, zellulärer Assays

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In den vergangenen drei Jahren ist die Häufigkeit multiresistenter Krankheitserreger in den Kliniken Deutschlands sprunghaft angestiegen. Diese bedrohliche Entwicklung erfordert eine Intensivierung der Forschungen zur Entwicklung neuer Methoden und Assaysysteme, die eine schnellere Detektion multiresistenter Keime und eine effektivere Wirkstoffsuche ermöglichen.

Im Rahmen eines Verbundvorhabens (iba Heiligenstadt, IPHT Jena, TU Ilmenau, HKI Jena, TILL Photonics GmbH und Impuls GmbH) wurde eine mikrosystemtechnische Plattform (SERIZELL) entwickelt, die eine hochparallele Erzeugung und Kultivierung von Mikrokulturen pro- und eukaryontischer Organismen und Zellen sowie ein Screening dieser Mikrokulturen ermöglicht. Das Wachstum der Mikrokulturen kann mit verschiedenen Methoden detektiert werden. Ausgewählte Mikrokulturen lassen sich aus dem System ausschleusen und einer weiteren Verwendung zuführen.

Hier wird ein tropfenbasierter Lebend-Zell-Assay zur Untersuchung des Einflusses von Wirkstoffen auf ausgewählte Mikroorganismen vorgestellt.

Darüberhinaus kann die Plattform für vielfältige Anwendungen in der Mikrobiologie, Biotechnologie und Analytik eingesetzt werden.

Das Vorhaben wird vom BMBF (FKZ 16 SV 1993 bis 1999 und 16 SV 2244) gefördert.

Miniaturisation of synthesis and screening assays

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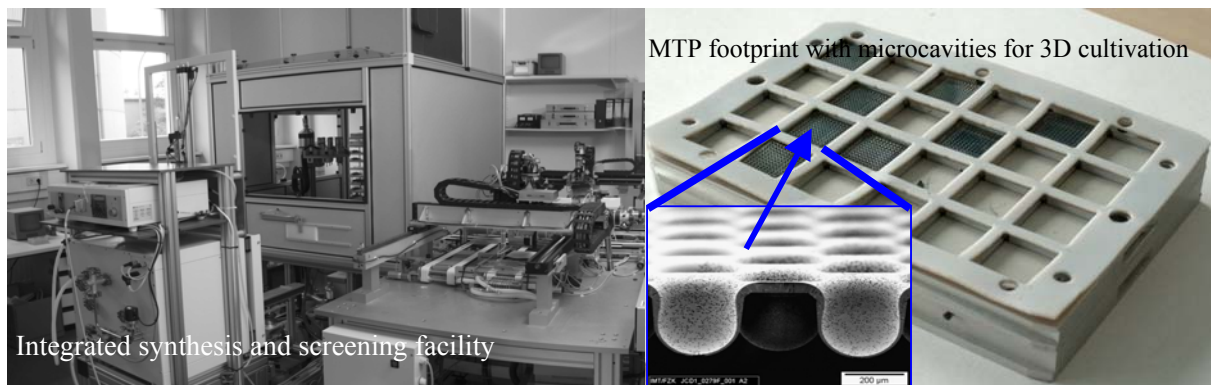
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Abstract: The idea of our approach is to shorten the drug discovery development chain by bringing organic chemical synthesis and biological testing closer together; so we constructed an integrated synthesis and screening facility. Here we present a case study of synthesizing a histone deacetylase (HDAC) inhibitor library based on solid phase synthesis in a spatially resolved “one bead one compound” approach. A library of hydroxamates has been synthesized, cleaved off the solid phase and transferred to an assay unit. Here the biological efficacy of the library (the IC₅₀ values and subtype-selectivity of some hits) has been determined.



As a result, combinatorial libraries can be synthesized and screened efficiently in one automated process, omitting long storage which could be costly as well as detrimental to the stability of the compounds especially in the case of reactive or labile substances. Not only the run-time was decreased significantly but also much of the chemical analysis normally needed for quality testing of the library after freeze-thaw cycles were diminished.

In addition one main topic of the talk will focus on the development and utilization of parallel, microfluidic 3D cell culture systems¹ with MTP footprint for early (HTS and follow-up) ADME/Tox testing. In this concept “follow-up” screens will be realized in such 3D parallel cell culture devices in an automated way. Furthermore the integration of new sensor systems for the application in drug screening will be discussed and first results will be presented.

¹ *The authors like to thank Stefan Giselbrecht, Eric Gottwald and K-F Weibezahn (Institute for Biological Interfaces, Forschungszentrum Karlsruhe, POB 3640, D-76021 Karlsruhe) for CellChips for 3D cultivation of Cell and fruitful discussions.*

B2

**Microfluidics for biomolecule and cell
techniques**

Bioreactor control using chip calorimeters

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Recently several authors have shown the possibility of detecting the metabolic heat production of micro-organisms held inside miniaturized, silicon chip based calorimeters. The activity of an ensemble of only ten mammalian cells was measured by JOHANNESSEN [1] using a micro-machined chip calorimeter cell of 720 pl volume. HIGUERA-GUISSET et al. [2] developed a special chip-calorimetric device to monitor the growth of a bacterial culture over several hours.

With a newly developed flow-through chip calorimeter system [3] we demonstrated the suitability of miniaturized calorimeters for its incorporation into technical relevant bioprocesses as a monitoring device. The calorimeter is sensitive enough to detect metabolic heat production rates in microbial suspensions of only a few micro-liters. Due to the small time constants a fast on-line operation in connection with bioreactors is possible even for aerobic processes. The high flexibility of the calorimetric systems enables the implementation of the calorimetric chip transducers into external technical systems.

As examples for the monitoring of aerobic and anaerobic processes, the growth of *Escheria coli* DH5 α and *Halomonas halodentrificans* CCM 286^T cultures, respectively, were calorimetrically determined [4]. Periodically, small quantities of bacterial suspension were taken out of a bioreactor and transferred into the calorimetric chip transducer of the system. The actual heat production rate of the bacterial culture was indicated with a time delay of less than one minute. The time dependence of the heat production rate was sufficiently described by established thermokinetic models and agreed very well with the off-line determined carbon-distribution of the assimilated carbon-substrate.

Furthermore, the feasibility of application of chip calorimeters for feed-back control in fed batch and chemostatic bioreactor operation modes will be discussed.

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Integrated Micro-opto-fluidic Systems for Optical Manipulation of Cell Cultures

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Abstract: Microfluidic systems have a large variety of applications in biomedicine and life sciences. The goal of the research is to develop integrated optofluidic microsystems which combine the microfluidic channels and parts of the optical functionality.

For most applications of microfluidic systems these systems need to be combined with electrical, optical or hybrid systems for analytics, sensing and micromanipulation [1]. The application of micro fabrication technology on the other hand has also triggered a revolutionary development towards microoptical systems integration. It is nowadays possible to monolithically integrate complex free-space optical functionalities [2].

Due to this compatibility in fabrication technologies it is straight forward to combine microoptically integrated optical elements with microfluidic channel systems for performance enhancement [3]. It is for example possible to integrate parts of the desired optical functionalities into the microfluidic system without significantly increasing the fabrication costs [4]. To this end the optical components are fabricated lithographically, e.g. as diffractive multilevel elements. The profiles are then subsequently replicated into the microchannel system fabricated e.g. in PDMS.

The resulting micro-opto-fluidic systems exhibit a well defined interface to the subsequent optical system. Fig. 1 illustrates the functionality of single optimized diffractive optical elements integrated into a microfluidic channel system. The goal of our experiments is to e.g. perform optical trapping and micromanipulation within the fluidic channel system. We investigate innovative solutions to overcome the geometrical constraints imposed by the fluidic systems and enable integrated solutions. Multifunctional diffractive elements integrated into the channel system, e.g. may provide additional focussing power to enhance 3D trapping and beam splitting for the generation of multiple optical tweezers. 3D trapping of microparticles is thus possible inside the channel system without the need of immersion objective lenses. Multiple optical traps generated by diffractive multiple beam splitter on the other hand allow the sorting and separation of various types of cells (Fig.1). We present the combination of these techniques with segmented flow systems and derive the potential for applications in bioanalytics.

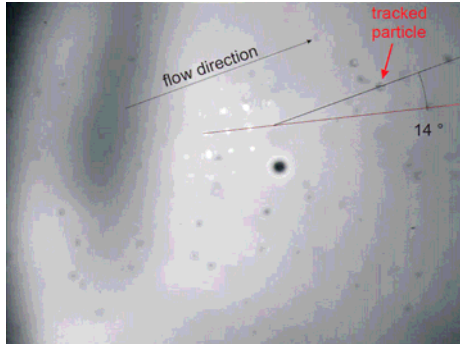


Figure 1: Particle deflection by a focus spot array

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Theoretische und rasterkraftmikroskopische Untersuchungen zur Kraftwirkung elektrischer Wechselfelder auf polarisierbare Partikel

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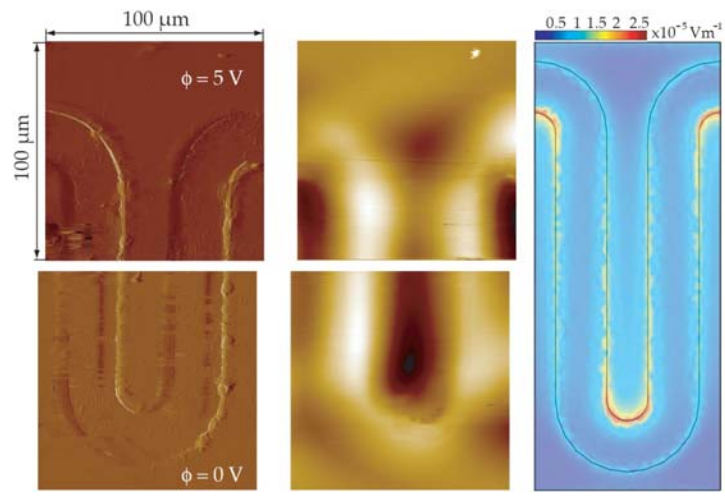
Wird ein polarisierbares Partikel in einem inhomogenen elektrischen Feld platziert, so erfährt es eine so genannte dielektrophoretische Kraft, die parallel zum Feldstärkegradienten gerichtet ist. Dielektrophorese eignet sich besonders gut zur markierungsfreien, selektiven Beeinflussung sowohl von künstlichen Partikeln als auch von lebenden Zellen.

Das notwendige elektrische Feld wird mit Mikroelektrodenstrukturen erzeugt, denn damit können hohe elektrische Feldstärken bzw. große Gradienten schon beim Anlegen kleiner Spannungen erreicht werden. Die Kenntnis der räumlichen Verteilung und der Größe des erzeugten elektrischen Feldes ist essentiell zur Berechnung der verschiedenen auf das Partikel wirkenden Kräfte.

Da keine exakte analytische Lösung zur Bestimmung des elektrischen Feldes und davon abgeleiteter Nebeneffekte für technisch relevante Elektrodenstrukturen existiert, würde eine umfassende experimentelle Abbildung aller tatsächlich bedeutenden Einflussfaktoren und deren Implementierung in ein FEM-Modell den anwendungsspezifischen Entwurf von Elektrodenarrays erheblich vereinfachen.

Die tatsächlich auftretenden Kräfte wurden also mit dem hochauflösenden Rasterkraftmikroskop nicht nur sichtbar gemacht, sondern auch simultan mit der Erfassung möglicher weiterer Einflussfaktoren orts aufgelöst genau quantifiziert. Dabei diente eine am Ende des Biegebalkens immobilisierte Polystyrenkugel als polarisierbares Volumen, auf welches eine dielektrophoretische Kraft wirkte, die wiederum den Biegebalken messbar auslenkte.

Anschließend wurden die mit dem erstellten FEM-Modell berechneten Ergebnisse mit den experimentellen Resultaten bezüglich ihrer Übereinstimmung verglichen.



Immunisierung mittels Mikrofluidik zur Therapie des Diabetes mellitus

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Die immunisierte Transplantation von Fremdgewebe oder Zellen, die therapeutisch wirksame Faktoren (z.B. Insulin, detoxifizierende Faktoren, gefäßprotective Faktoren, etc.) produzieren, wird völlig neue, patientenfreundliche und stark Kosten senkende Wege in der Therapie von Stoffwechselerkrankungen (z.B. *Diabetes mellitus*) eröffnen. Ein Schutz vor den Komponenten der immunologischen Abwehr des Patienten lässt sich durch Verkapselung in (mit zweiwertigen Ionen vernetzten) 3D-Alginatmatrizen [1] erreichen, so dass auf eine Applikation von Immunsuppressiva verzichtet werden kann. Erforderlich dafür sind mikrofluidische Systeme, die einige Vorteile bieten verglichen mit konventionellen Techniken. Die Erzeugung von einzeln isolierten, zellhaltigen Medienkompartimenten innerhalb eines abgeschlossenen Systems ist automatisiert möglich, indem unter Nutzung geeigneter mikrostrukturierter Fluidikchips definierte Volumina an Medium in einen Trägerstrom eines nicht mischbaren, unpolaren Trägermediums eingebracht werden [2]. Die Erzeugung von Medienkompartimenten ist über einen weiten Bereich im Nanolitermaßstab reproduzierbar möglich.

Durch den Einsatz von mikrofluidischen Systemen, Spritzenpumpen, Mikroventilen, Massendurchfluss- und Druckregelungskomponenten wird ein geschlossenes System entwickelt, mit dem Mikrotransplantate GMP-Konform und mit einem hohen Durchsatz generiert werden können. Das Augenmerk liegt hier bei der Langzeitstabilisierung der Mikrotransplantate, um so eine Alternative zur Behandlung von *Typ-1-Diabetikern* zur Insulin-Verabreichung zu erreichen und die Folgeschäden des *Diabetes mellitus* zu verhindern.

Im Vortrag sollen Aussagen zur gewählten methodischen Vorgehensweise, deren Umsetzung und zu ersten Ergebnissen getroffen werden.

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Sterilisierbares Mikroventil mit PNIPAAm-Hydrogelaktuator für Lifescience-Applikationen

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Am Beispiel der Entwicklung eines Mikroventils auf der Basis eines PNIPAAm-Hydrogelaktuators (Abbildung) werden die Anforderungen und Randbedingungen für die Applikation von Mikrosystemen in den Lebenswissenschaften dargestellt. Die Aufbau- und Verbindungstechnik zur Herstellung des Mikroventils mit besonderem Augenmerk auf die technologischen Probleme der MST-Massenfertigung wird beschrieben. Exemplarisch werden Ergebnisse einer Applikation des Mikroventils in der Biotechnologie vorgestellt. In dieser Applikation wird ein Array, bestehend aus 6 Mikroventilen, für die automatische Probenahme aus einem in einer Mikrotiterplatte ablaufenden biotechnologischen Prozess eingesetzt.

Folgende Schwerpunkte werden behandelt:

- **Verwendete Prozessierungstechniken**
- **Aufbau- und Verbindungstechnik**
- **Fragen der Sicherheit bei biomedizinischen Applikationen (Sterilisierbarkeit, Aufrechterhaltung der Sterilität)**
- **Minimierung des manuellen Aufwandes für Vorbereitung, Kultivierung und Detektion**
- **Minimierung des Kulturvolumens und eventuell benötigter Reagenzien für die Detektion**

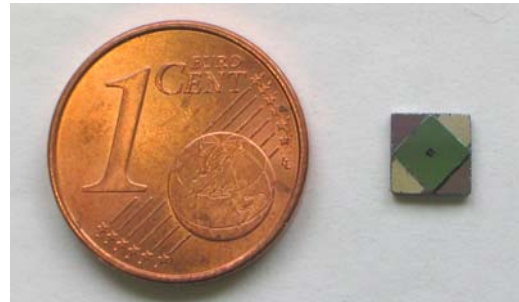


Abb.: Mikroventil im Größenvergleich

Mit dem vorgestellten Mikroventil steht ein MST-Aktuator zur Verfügung, der die spezifischen Randbedingungen für Anwendungen in den Lebenswissenschaften berücksichtigt und erfüllt. Die Ergebnisse lassen darüber hinaus Applikationen auf anderen Gebieten wie der Umwelttechnik und der Lebensmitteltechnik erwarten.

Module zum simultanen Mischen und Prozessieren von Suspensionen in der Mikrofluidik

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Die Verwendung von mikrofluidischen Systemen ermöglicht das Arbeiten mit wässrigen Kompartimenten im segmentierten Fluss. Diese Kompartimente können aus Kulturmedien bestehen und reproduzierbar in großer Zahl im Nanoliterbereich erzeugt werden. Eingebettet sind sie in einem unpolaren Trägerstrom einer nicht-mischbaren Flüssigkeit; die Kultivierung von Mikroorganismen in diesen Kompartimenten wurde bereits gezeigt [1].

In einem solchen mikrofluidischen System können einzelne Kompartimente zuverlässig adressiert werden, was die kontrollierte Zugabe gelöster Substanzen ermöglicht. Eine Gradientendosierung erlaubt so die Erprobung von Wirkstoffkollektionen über einen weiten Konzentrationsbereich. Auch ist die Einbringung von Beads mit angekoppelten Molekülen möglich, die vorher durch gezielte Festphasensynthese kovalent an diese Träger gebunden wurden. Bei der Durchführung von Assays mit Mikroorganismen oder tierischen Zellen muss jedoch gewährleistet sein, dass zu Beginn einer Testreihe die Kompartimente vergleichbare Zelltitel mit hohen Vitalitäten aufweisen. Unter dieser Voraussetzung können später beobachtete Wirkungen der eingeschleusten Substanzen miteinander in Beziehung gesetzt werden. Während unserer Arbeiten im Projekt SERIZELL [2] entwickelten wir zwei Mischmodule, mit denen zum einen Zellsuspensionen gemischt und zum anderen mit frei wählbaren Fließraten in mikrofluidische Kapillaren prozessiert werden können. Die Eignung beider wurde mit Suspensionen von Hefen und Hybridomzellen belegt, indem das Mischverhalten und die sich ergebenden Vitalitäten nach Durchmischung und Transport untersucht wurden. Hybridomzellen konnten bis zu einer Stunde im Gefäß konditioniert werden, ohne dass ihre Proliferationsfähigkeit beeinträchtigt wurde.

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B3

**Multicellular Systems and
toxicological monitoring**

Cryomicrotechnology: enabling technology for vaccine research and regenerative medicine

Heiko Zimmermann

It is well accepted that the development and establishment of new cell based therapies will rely on safe sources of human cells. An ongoing approach to achieve this is the construction of high quality biobanks (www.biobanks.eu). Not so well known is the need for cell banks by the developers of new vaccines against epidemics (e.g. AIDS) which has led to initiatives like the “GHRC”-project for setting up a global HIV vaccine research cryorepository (www.ghrc.de). Banked immune cells as well as infected cells which serve as a substrate for in vitro vaccine trials (see image). Another major project is the “Crystal”-project, an international approach to standardise and optimise methods, technology and protocols for the banking of stem cells of all sources for therapeutic purposes (www.crystal-eu.org).

These examples indicate the recognised importance of high quality preservation of (primary) cells using the only widely accepted method for life storage of cells, i.e. freezing and storage at ultra low temperatures (below minus 130°C). As freezing is no natural process and highly stressful for the cells, new approaches have to be done to overcome the physical and biological limitations for successful cryo preservation of sensitive cells and cellular systems.

A promising approach in this scientific field is the use of micro technology to allow more defined freezing regimes as well as optimised addition and removal of cryo protectant agents (like the unloved but widely used cytotoxic DMSO) resulting in new cryo protocols. The successful integration of microelectronics and sensors suitable to operate robust in the extreme environment of a liquid nitrogen cooled cryo tank has made the vision of smart cryo micro systems appear only a few steps apart. Cryomicrotechnology enabled cryo banks promise to solve the remaining biophysical and logistical problems, especially mismatch-free sample storage, data handling, and world wide sample controlled preparation workflows.

The talk will summarise the political and economic background and the pan-European situation of cryo banking. Basic cryobiological problems are described and the state of major European and trans-Atlantic projects is reported. The talk will focus especially on the application of micro systems and micro electronics to the field of cryobiology. The new standard technology platform for HIV related sample storage will be shown (see image).



Einsatz eines Mikrofluidsystems zur Durchführung toxikologischer Studien an Mikroorganismen

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Eine ständig wachsenden Anzahl an technisch eingeführten potentiellen Schadstoffen lässt der Frage der Risikoabschätzung eine immer bedeutendere Rolle zukommen. In vielen Forschungsarbeiten zur Bewertung der Toxizität wurde vorwiegend nur von der Wirkung einzelner Substanzen ausgegangen. Die Realität zeigt aber, dass Mensch und Umwelt oft nicht nur einer einzelnen Substanz ausgesetzt sind, so dass die Bewertung von Schadstoffkombinationen immer bedeutender wird^{1,2}. Die Komplexität dieser Untersuchungen erfordert den Einsatz neuer Methoden, mit denen Screenings breiter Parameterfelder möglich werden.

Im Rahmen dieser Arbeit sollte durch Einsatz eines Mikrosystems die Wirkung verschiedener toxischer Stoffe als auch Stimulanzen auf das Wachstum von Mikroorganismen untersucht werden. Dafür wurde die Methode des segmentierten Flusses angewandt, bei welcher durch die Nichtmischbarkeit zweier Phasen eine große Anzahl abgetrennter und in ihrer Zusammensetzung variierender Reaktionsräume (Mikrofluidsegmente) geschaffen werden können.

Die Versuchsanordnung bestand aus einem ansteuerbaren Spritzenpumpensystem mit sechs Dosiereinheiten (*Cetoni GmbH*), einem 7-Port- Manifold als Segmentierungsmodul (*PEEK, Upchurch*) und Schlauchspulen (*PTFE-Schlauch, 0,5mm ID, Bohlender GmbH*) für die Lagerung. Zur Charakterisierung der erzeugten Segmente wurde zum jeweiligen Wirkstoff ein Farbstoff (*Cochinillerot A*) zugesetzt. Die Detektion erfolgte direkt durch den Schlauch (*Teflon FEP, 1/16 Zoll ID, Upchurch*) über zwei separate Mikrodurchflussphotometer. Für die Konzentrationseinstellung der toxischen Substanzen im Segment und die Datenanalyse standen eigens geschriebene LabView-Programme zur Verfügung.

Als Modellorganismus wurde *Escherichia Coli (RV 308, HKI Jena)* eingesetzt, welcher 24h bei 37°C und 80% Luftfeuchte im Segment kultiviert wurde. Die Messung mittels Photometer erfolgte zum Zeitpunkt 0h und nach 24h. Zur Bestimmung der Farbstoffkonzentration in den Fluidsegmenten wurde eine LED mit 505nm Wellenlänge und der Trübung eine LED mit 605nm eingesetzt. Als Wirkstoffe wurden Polyvenyrolidon (PVP), 2,4-Dichlorphenol (DCP), Dimethylsulfoxid (DMSO), Adrenalin, Coffein und die Kombination von DCP und Adrenalin eingesetzt.

Mit den Versuchen konnte eine Abhängigkeit des Wachstums von *E. coli* mit Änderung der Konzentration an Schadstoff gezeigt werden. Weiterhin ist es mit dieser Methode möglich, spezifische Dosis-Wirkungskurven für die entsprechenden Substanzen zu erstellen. Eine Zellzählung für jede Konzentrationsstufe nach Ausbringung der Segmente aus dem Schlauch, erlaubte eine Aussage über den Zustand der Organismen und die Zelldichte nach 24 Stunden Kultivierung. Außerdem wurden Vergleichsversuche in Mikrotiterplatten (MTP) durchgeführt und mit den aus den Segmenten gewonnenen Ergebnissen verglichen. Dabei zeigte sich, dass die in MTPs und in Segmenten gewonnenen Dosis-Wirkungskurven vergleichbar sind.

Die vorgestellte Technik erfüllt eine wichtige Voraussetzung für die Realisierung von Multiparameter-Screenings, d.h. die Erzeugung und Auswertung einer großen Anzahl verschiedener Parameterkombinationen, mit deren Hilfe bessere Analysen unterschiedlichster Effekte von Umweltparametern auf biologische Systeme erreicht werden können.

Biomikrosysteme zur Kultivierung von Indikatororganismen für Umwelt-Monitoring, Gefahrstoffdetektion und Zytodiagnostik

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In Zeiten des Klimawandels rückt die Ressource Wasser zunehmend in den Blickpunkt der Anstrengungen zur nachhaltigen Nutzung von Lebensgrundlagen. Trink- und Brauchwasser werden besonders in den wärmeren Regionen der Erde und in Ballungszentren immer knapper. Somit gewinnt die Analyse von Wasser insbesondere für das Umwelt-Monitoring immer mehr an Bedeutung. Mit ihrer Hilfe kann man chemische, physikalische und mikrobiologische Eigenschaften von Proben untersuchen und somit Abweichungen in der Wasserqualität detektieren.

Für klassische Wasseranalysen kommen verschiedene, vom Gesetzgeber vorgeschriebene Verfahren (AbwasserVO des WHG) zum Einsatz. Neben organoleptischen Charakteristika (Geruch, Färbung, Trübung, Bodensatz) und physikochemischen Grundparametern (pH-Wert, Sauerstoffgehalt, Phosphatgehalt etc.) die direkt vor Ort gemessen werden, sind auch mikrobiologische Untersuchungen mit Hilfe von Indikatororganismen vorgeschrieben. Dabei werden prinzipiell zwei Varianten unterschieden. Gewässer- und Schlammproben werden auf das Vorhandensein, bzw. Fehlen bestimmter Organismen (Zeigerarten) untersucht. Diese Verfahren werden bei natürlichen Gewässern eingesetzt. Für fließende Gewässer gilt der Saprobienindex, welcher die Zuordnung zu Güteklassen ermöglicht. Für stehende Gewässer analysiert man das Nährstoffangebot eines Standortes (Trophiestufen) [1].

Für künstliche Gewässer wie zum Beispiel Schwimmbäder, Wasseraufbereitungsanlagen u.ä. kommt die beschriebene Variante nicht in Frage, da hier aufgrund des hohen Reinheitsgrades des Wassers keine Indikatororganismen vorhanden sein sollten. Daher entnimmt man Wasserproben, bringt Testorganismen ein und beobachtet deren Reaktionen. Hierbei kommen Arten zum Einsatz, die über eine geringe Indikationsbreite verfügen (DIN 38412) [2] Diese Tests sind mit großem labortechnischen Aufwand verbunden. Die verwendeten Populationen sind nicht isophasisch, somit lassen die Ergebnisse nur eine grobe Aussage über den Einfluss auf die Vitalität zu.

Zur Vereinfachung und Verbesserung der Analysen wird ein mikrosystembasiertes Frühwarnsystem für Schad- und Gefahrstoffe entwickelt. Dazu sollen kleine, standardisierte, robuste und preiswerte Mikrosysteme (Bio-MEMS) entwickelt werden, die es erlauben, eine verteilte Sensorik in der Peripherie der Versorgungsnetze zu etablieren. Für das Testsystem wurde eine Funktionszelle entwickelt, die den Anforderungen für die Zelltests entspricht. Die Funktionszelle eignet sich nach ersten erfolgreichen Labortests zur Langzeitvitalhaltung (>48h) ein- bzw. mehrzelliger Indikatororganismen (z.B. *Scenedesmus*, *Tetrahymena*, Leuchtbakterien, *Daphnia*). Dabei können neben der visuellen Bestimmung toxisch signifikanter Aktivitätsmuster (z.B. Erkennen von Bewegungen, Formänderungen) auch andere zellphysiologische Parameter mit Sensorik in der Mikrokavität detektiert werden. Morphologisch sichtbare Reaktionen eines Organismus in unbelastetem und belastetem Medium im Mikrosystem können aufgezeichnet werden.

Um die volle Funktionalität für aussagekräftige Testverfahren zu erreichen, bietet es sich an, diese Funktionskammern derart zu kombinieren, dass mehrstufige Tests möglich werden. Das kann in Form von Kaskadierung und kombinatorischer Fluidlogik geschehen. Mit Hilfe des vorgestellten Systems wird die in situ-Beobachtung einzelner Organismen oder einer definierten Population und Zellkulturen in Mikrosystemen ermöglicht. Es können vor-Ort- und Echtzeitmessungen unter kontinuierlicher mikroskopischer Beobachtung durchgeführt werden, da der Bewegungsbereich der Objekte auf das Bildfeld und die Fokusebene begrenzt ist. Somit wird es möglich, Verunreinigungen zu detektieren und adäquat darauf zu reagieren.

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The pipe based bioreactors : An innovative high-throughput bioreaction platform with a wide spectrum of analytical applications

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The fast-growing demand of variable measurement systems for the industrial branch and also for research institutes is the starting point to establish a new easy-to-use platform accomplishing biotechnological challenges. This system should be adaptable for a large variety of applications, highly efficient, accurate, low-cost and only need a small amount of reagents per assay.

This lecture will introduce the *pipe based bioreactors (pbb)* platform with various examples of applications. The Institute for Bioprocessing and Analytical Measurement Techniques (iba) in Heiligenstadt endeavours to use small fluidic droplets with a biological or chemical origin in the nanoliter scale to create bio- or chemical reactors under special conditions. The main principle is to use segmented flow with water soluble samples that can contain cells or chemical compounds. With the help of a special microchip such solutions will be embedded as fluidic droplets into a carrier fluid of a non-polar organic liquid.

One advantage of this bioreaction platform is statistical reliability. The maintenance of hygiene and sterility can be easily warranted. Additionally, as this system is closed, it can protect laboratory employees against potentially harmful samples. The transportable reactors (fluidic droplets) allow a high flexibility of the system and open possibilities for new applications. Due to the basic composition of the system there is a possibility for low-cost commercialization of this special high-throughput system. The feasible applications reach from medicine (e.g. metabolic disease), environmental techniques (e.g. bioremediation of oil) to food technology (e.g. contamination of food). These efforts need a further application of measuring methods to the *pipe based bioreactors* system.

First results were achieved by using this *pipe based bioreactors* as a high-throughput individual cultivation system in a cooperation project called MINIKULT® [1]. Isolation of rare microorganisms from soil samples were successful. The system reached a capacity of 400000 samples per day. The next developing step of *pbb* was realized in the SERIZELL project to detect cells in fluidic droplets after cultivation and to separate them after this procedure to store in a tube for other investigations. Additionally, in this project an important tool named MiniCellMix® was developed to mix cells and beads in a gentle way to get reproducible injections of these components into the droplets [2]. The preliminary research with this *pipe based bioreactors* system was the background for a new concept which describes a new rapid detection of microorganism in food. The procedural steps from the biomagnetic separation of cells until the detection and quantification of germs should not exceed eight hours in total.

Finally, research in the area of microsystem applications by adapting measuring techniques to biological environments and in bioprocessing techniques will allow

successful introduction of this platform to various analytical efforts in different applications.

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F
Sensorics, Arrays and Analytical
Microsystems

Bioanalytische Mikrosysteme für die Labordiagnostik

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Die vorgestellten bioanalytischen Mikrosysteme bestehen im wesentlichen aus zwei grundlegenden Funktionseinheiten. Einem (Bio-)Sensorarray das als (multi)sensorisches Element fungiert und einer Mikrofluidik, die entweder aus einfachen Kapillaren besteht oder - in komplexerer Ausführung- die Mischung einer Probelösung mit einer Reagenzlösung sowie deren Zuführung zum Biosensorarray übernimmt. Die Mikrosysteme werden in einer Laminattechnologie aus photostrukturierbaren Trockenresist-Folien aufgebaut, auf die bei Bedarf auch Dünnschichtelektroden aufgebracht werden. Alternativ werden in einer Hybridtechnologie Glaschips auf Polymerkanäle gebondet. Die bis zu fünf verschiedenen Biosensoren im Biosensorarray pro Chip werden durch automatisiertes Dispensieren von jeweils ca. 5 Nanolitern der gewünschten Enzymmembran auf die entsprechende Elektrode hergestellt. Die ökonomische Aufbringungsart erlaubt sowohl die Herstellung kostengünstiger Sensoren mit teuren Enzymen als auch die flexible Belegung des Biosensorarrays entsprechend den Anforderungen an die Analytik. So wurde z.B. für das Monitoring von Zellkulturfermentationen ein Biosensorarray mit Glukose-, Laktat-, Glutamin- und Glutamat-Biosensoren hergestellt, für die Transaminasenmessung solche mit lediglich Glutamatsensoren und für medizinische Anwendungen üblicherweise redundante Chips mit zwei Glukose- und Laktatsensoren. Die Enzymmembranen selbst sind Gegenstand intensiver Forschung, die Aufschluss über spezifische Proteinstabilisierung gibt, aber auch Wege zu neuen Biosensoren aufzeigt.

Mit einer Mikro dialysesonde gekoppelt wurden die BioMEMS erfolgreich an menschlichen Probanden zum Stoffwechsel-Monitoring im Fettgewebe eingesetzt. Als Weiterentwicklung wurden miniaturisierte Mikro dialysesonden direkt in das Mikrosystem integriert.

Möbius-Mikromischer erlauben das Zumischen von Reagenz zur Probe und erweitern dadurch die Analytenpalette auf ein Vielfaches der Anzahl der verfügbaren Biosensoren. Als Beispiel sei die Ammoniummessung in Speichel von Kindern angeführt, das die schmerzhafte Blutabnahme ersetzen soll. Bei den Immunoassays dienen Mikrokanäle als Reaktor-Säulchen. Das günstige Oberflächen /Volumenverhältnis in den Kapillaren ermöglicht schnelle und empfindliche Tests für Marker in der Notfallmedizin wie z.B. Troponin. Ein vollständig folienbasiertes BioMEMS für die Durchführung dieser elektrochemischen Kapillar-Immunoassays wurde realisiert. Neben der oben erwähnten Anwendung wird das Mikrosystem beispielsweise in der Serologie von Hepatitis und zum Nachweis von Substanz P eingesetzt.

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Requirements for sensitive detection of biologically relevant macromolecules

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Detection of biologically relevant macromolecules like antigens or cytokines needs sensitive and specific techniques. Currently, Western Blot, ELISA and perhaps Immuno-PCR are the methods of choice in diagnostics. Capillary Immune Electrophoresis is under development and needs some refinement. Most of these methods fulfill stringent criteria with respect to sensitivity and specificity i.e. to find all sick cases (no false negatives) and to find all healthy ones (no false positives). A major aim of all techniques focuses on the level of detection. Antigens as an example are present in varying amounts in sera. Patients infected with Hepatitis B virus may harbour 10^4 – 10^9 virus particles per ml serum. A certain number of molecules, e.g. a specific antigen, has to be present in order to become detectable in the respective assay format like an ELISA. Many times picogram amounts can be detected and the concentration of the same antigen can be extrapolated to define its molarity in serum or blood. Confusion exists when gram, mol and molarity are incorrectly used. Another goal of recent innovative developments refers to miniaturize assay systems like Lab-on-a-Chip strategies. However, if one calculates the absolute number of molecules, their microgram amount or the molarity in the source material then one realizes that diminishing the size of an assay format may not lead to improve on sensitivity. To circumvent this disadvantage new labelling and detection techniques are required. To illustrate the needs for “*small and sensitive*”, examples from infection pathology like prion protein, Hepatitis B- and HI-viruses will be presented.

Einsatz von Farbsensoren in der Mikroanalytik

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Farbsensoren werden heutzutage für spezialisierte Anwendungen wie z.B. spektrale Untersuchungen immer wichtiger. Es werden Konzepte und Messsysteme entwickelt, die in der Mikroanalytik unter Verzicht auf Monochromatoren, Farbsensorarrays mit optimierten Wellenlängen als Detektoreinheit benutzen. Die Entwicklungen reichen von Geräten zur Auflichtmessung bis hin zu höchstempfindlichen miniaturisierten Spektrometern für Fluoreszenzuntersuchungen im Bereich der Umweltanalytik. Der Vorteil derartiger Messsysteme liegt neben der Kosten- und Messzeiterparnis insbesondere in der Miniaturisierung und in der damit problemlosen Implementierung solcher Messsysteme in der Produkt- und Produktionskontrolle.

Vorteile der Farbsensoren gegenüber anderen Detektoren sind einerseits deren minimale Abmessungen, als auch andererseits ihre Informationsdichte aufgrund der Tatsache, dass sie statt nur einer gemessenen Intensität einen Farbwert liefern, der die analytische Auswertung der erhaltenen Signale wesentlich verbessert.



Abb. 1: RGB- und True-Color-Farbsensoren „JENCOLOR“

Üblicherweise bestehen diese Farbsensoren (z.B. „True-Color-Sensoren“) aus 19×3 Fotodioden die auf einem Chip integriert sind. Die Dioden werden als Elemente einer hexagonalen Matrixstruktur (Abb. 1 rechts) ausgeführt, welche einen Durchmesser von 2 mm hat. Um ein geringes Übersprechen zwischen den Dioden zu erreichen, werden sie durch separate Strukturen voneinander getrennt. Jede Fotodiode ist mit einem dielektrischen Spektralfilter versehen, um im CIE-Farbraum zu arbeiten.

Im Vortrag sollen typische Anwendungen von True-Color-Sensoren in der Mikroanalytik gezeigt und deren analytische Verwendbarkeit bis zum Aufbau von miniaturisierten Spektrometern diskutiert werden. Grenzen und Vorteile solcher Systeme werden anhand eigener Arbeiten diskutiert.

**On-line quantization of organic analytes by
micro flow-through
Surface Enhanced Raman Spectroscopy(SERS)**

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The recent development of microfluidic platforms for online monitoring of analyte concentrations by surface enhanced Raman scattering (SERS) has qualified Raman spectroscopy as valuable tool for highly sensitive lab-on-a-chip applications. Due to the high sensitivity of SERS very low detection limits down to a few hundred molecules[1] have been reached. Advantages of droplet based microfluidics have been successfully used for online monitoring of fluctuations of drug concentrations[2,3]. Therefore, all the required steps of sample preparation, including injection of analyte solution, dosing of nanoparticle suspension and aggregation reagent to the sample droplets are integrated into a single, optical transparent, all-glass microfluidic chip device. The optimum retention time between start of the aggregation process and the optical readout strongly depends on the rate of formation of nanoparticle/analyte-conjugates. For investigation of this process a transparent residence channel for measurement of Raman spectra at different residence times was integrated.

Here, we will give an introduction to the developed platform for micro analytics, its application for on-line monitoring of drug concentrations and discuss first results on investigation of aggregation kinetics.

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On-Chip PCR: Technology and Prospects

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The polymerase chain reaction (PCR) is an enzymatic, thermally controlled process which specifically amplifies nucleic acids (DNA) with a high yield [1-2]. Because of the mechanistic simplicity of the PCR, this process was an ideal candidate for miniaturization during the last decade [3-6].

Based on the stationary functional principle of the conventional thermocyclers, the development of a chip based device was pursued at the IPHT Jena. These chip devices are produced with wet chemical etching processes and photolithographic structuring technologies in order to realize the heater and sensor structures on glass substrates. A real-time detection of the amplicons is realised by a coupling of an optical fibre in connection with a excitation- and detection unit. Using fluorescent based dyes and respectively probes, PCR processes on the chip down to a sample volume of only 0.5 µl could be still detected.

Through the powerful use of the potential of the micro system technology a stationary chip thermocycler with integrated real-time detection and low power consumption could be realized. This system enables PCR at short analysis times and low sample volumes.

This accomplishment represents a key step towards the realisation of a technological platform for on-chip real-time PCR in point of care diagnostics.

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