Inkjet Printed Structures for Smart Lab-on-Chip Systems

Erik Beckert[1], Oliver Pabst[1,2], Zhe Shu[1,2], Christin Herzog[4], Stephan Mittwoch[1], Jolke Perelaer[3], Ulrich S. Schubert[3], Ramona Eberhardt[1], Andreas Tünnermann[1,2], Stefan Nagl[4], Detlev Belder[4]

[1] Fraunhofer Institute for Applied Optics and Precision Engineering, Jena, Germany

[2] Friedrich-Schiller-Universität Jena, Institute of Applied Physics, Abbe Center of Photonics, Jena, Germany

[3] Friedrich-Schiller-Universität Jena, Laboratory of Organic and Macromolecular Chemistry, Jena, Germany

[4] Universität Leipzig, Institute for analytic Chemistry, Leipzig, Germany

Abstract

Lab-on-Chip microfluidic systems require onboard functionalities such as fluid pumping, heating and fluorescent or chemical sensing. An approach is presented to realize these structures by inkjet printing of functional materials or material sandwiches and subsequent curing or sintering. In detail an electro-active polymer membrane pump with passive micro-structured valves, an open loop controlled ohmic heater structure for maintaining discrete fluid temperatures and electrodes for free-flow isoelectric focusing along with fluorescent hydrogel pads for pH sensing are discussed that all together are printed onto the cover foil of a polymer based microfluidic chip platform at the size of a microscope slide.

Introduction

Microfluidic lab-on-a-chip (LoC) systems are miniaturized microchip-sized laboratories that are used to control, mix and analyze small fluid volumes in order to carry out various biological and chemical analysis processes. LoC can be fabricated from polymer materials in a very cost efficient manner and usually consist of a chip substrate with fluid channels and a cover foil, enabling single use disposables at the Point-of-Care (PoC). For certain analysis tasks the chip also has to be equipped with functional structures such as electrode-like wirings. This can lead to higher complexity and also more and expensive additional lab equipment to drive and use the LoC. The goal for polymeric LoC thus is to introduce more functionality by simple and low-cost manufacturing processes into the chip while maintaining simple interfaces that do not require additional lab devices. Inkjet printing is a promising technology; it allows an easy manufacturing of the usually planar structures required for LoC. Examples for several realized functionalities by printing the respective material or material sandwich will be given in the following chapters. Basis for these is mostly an electrically conductive wiring, in addition piezoelectric or pH sensitive polymers are used.

Heaters for Fluid Temperature Control

Local heating is required for certain chemo/bio-reactions within microfluidic channels. A prominent example is the polymerase chain reaction (PCR), which typically clones DNA sequences by thermo-cycling them between 60 °C and 95 °C. By inkjet printing ohmic heaters on top of microfluidic channels, a most direct and controlled heating of the fluid within the channel is possible [1]. Furthermore, by drying and partial sintering of inkjet printed silver nanoparticle dispersions, a controllable and relatively high resistance can be obtained, which eliminates using specific high resistance and expensive materials. Nevertheless, it is required to obtain a defined and homogeneous temperature over a specific area (normally a part of micro-channels) at relatively low driving powers; another requirement is to achieve fast heating and cooling times in order to fulfill the chemo/bio-reaction requirements.

In order to investigate the influence of the geometry parameters on heating homogeneity, five different wiring structures with different line width and line spacing combinations but a same heating area (15 × 10 mm²) were inkjet printed using a silver nanoparticle dispersion (NPS-JL, Harima Chemicals, Inc.) on glass substrates. Fig. 1 and Tab. 1 illustrate the structure designs and line width and spacing parameters.

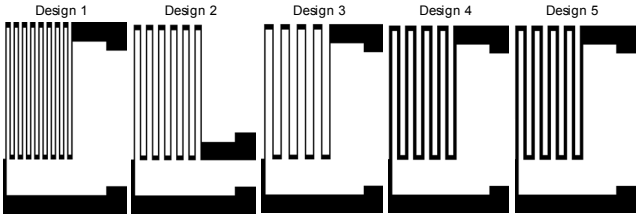

***Figure 1**. Five different structural designs of meandering conductive heaters with a same heating area*

Table 1. Design parameters for Design 1 to 5

Design	1	2	3	4	5
Line width in design / mm	0.3	0.3	0.3	0.6	0.9
Line spacing in design / mm	0.3	0.6	0.9	0.6	0.6

After thermal sintering at 200 °C for 60 min, the structures revealed a conductivity of approximately 45 % of bulk silver. The different designs have different resistances mainly due to different numbers of meandering lines and, consequently, lead to different temperatures caused by ohmic losses. With the help of an IR thermal camera the thermal distribution on the backside of heater samples was imaged and analyzed at elevated temperatures and by driving the printed heaters at different voltages, the average temperature over defined heating areas vs. driving power was compared between the different designs. According to Fig. 2, design 5 with relatively large line width and low resistance

requires the highest driving power to heat up to the same temperature, but it shows the lowest standard deviation of <1 K and thus the best homogeneity for a defined area, fulfilling the requirements for biochemical reactions such as PCR.

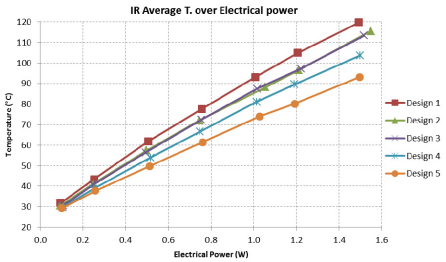

***Figure 2**. Average temperatures over whole heating area vs. driving electrical power for different designs*

In order to investigate heating and cooling rate, the best performing design 5 was selected for a dynamic investigation. 60 °C and 95 °C were chosen for the low and high thermal phase required e.g. for a PCR amplification. As depicted in Fig. 3 (right), the measurement procedure was: first heating up to 60 °C and wait until the temperature stabilizes; then shifting the driving current/ voltage to a high level (P3) in order to increase the heating rate; afterwards reduce the driving current/voltage (P2) when the temperature is approaching 95 °C; after 10 min waiting, the input is switched off until the temperature approaches 60 °C; finally a low driving level (P1) is used to maintain a temperature of 60 °C. The average temperature measured by the IR thermal camera is shown in Fig. X (left). The calculated heating rate is ≈2.9 K/s and the cooling rate is ≈1.16 K/s. Compared to the requirements of heating rate > 4.5 °C and cooling rate > 3.5 °C for a PCR, the heating rate can be achieved by further increasing the heating power while fast cooling requires extra cooling components.

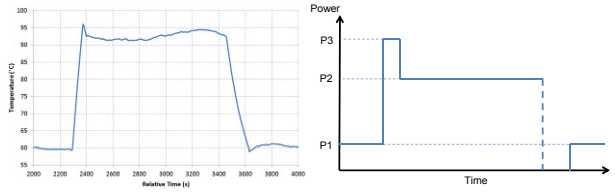

***Figure 3**. Time dependence behavior of the average temperature generated by design 5 (left) at different driving conditions (right) for heating and cooling rate investigation*

Capacitive Fluid Presence Detectors

Electrodes for capacitive structures on top of a fluidic channel that use the absent / non-absent fluid as a varying dielectric media of the capacitive structure are an alternative to complex other fluid detection methods such as light barriers. In a most simplified setup, the polymer cover foil of the fluid channel can be used as permanent dielectric part of the capacitor, while the fluid in the channel acts as the varying dielectric part.

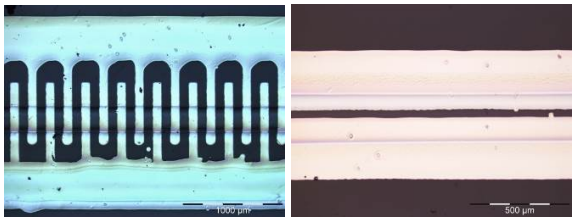

***Figure 3**. A horizontal fluidic channel with interdigital capacitor electrodes (left) and plate capacitor electrodes on top*

Two configurations incorporating interdigital electrodes and normal plate capacitor electrodes are shown in Fig. 4, each on top of a 200 µm wide 200 µm deep channel. The base capacity of both configurations, normalized to the length of the capacitor on top of the channel, is 0.02 pF/mm to 0.05 pF/mm for the plate and 0.08 pF/mm to 0.14 pF/mm for the interdigital electrode capacitor. While the base capacity is important to design the capacity measurement system in general, for the absence/ presence detection of a fluid the change in capacity is more relevant. Tab. 2 illustrates the range of capacity changes, if the dielectric is either air or an aqueous fluid. The capacity change is also normalized to the length of the capacitor over the fluid channel.

Table 2. Capacity change for air to aqueous liquid filling of the underlying channel

Capacitor Type	ΔC	ΔC/L
Interdigital	0.13 pF – 0.17 pF	2.4 pF/m – 3.2 pF/m
Plate	0.29 pF – 0.42 pF	6.0 pF/m – 8.7 pF/m

Assuming standard LCR-meters with 0.01 pF resolution and a smallest and safe detectable capacity change of 0.04 pF for the plate capacity like structure a minimal length of the sensing structure >4.5 mm can be calculated. This value renders to capacitor useful for common and “not so dense” LoC chips, while the performance still can be enhanced by placing the capacitor structure closer to the fluid channel (e.g. o the face-down side of the cover foil, printing an additional dielectric layer over the electrodes, which can be much thinner than the cover foil itself).

Electrodes for Free-Flow Electrophoresis

Free Flow Isoelectric Focusing (FFIEF) is the electrophoretic separation of biomolecules in a fluid containing a pH gradient and under the influence of an electric field (Fig. 4), using their electromobility. In a microfluidic chip, the µFFIEF is one of the major tasks to be performed for the separation of analyte ingredients before their detection. Electrodes are required therefore at the corners of the separation chamber in order to apply the electric field throughout the fluid under investigation [2].

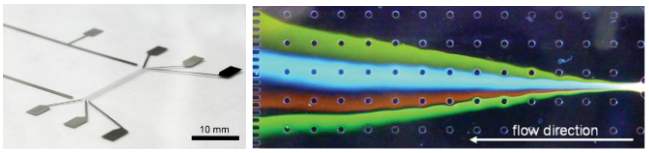

***Figure 4**. Printed electrodes (left) and detail of an electrophoretic separation of four fluorescent dyes (right)*

Printed electrodes to be used for FFIEF in LoC have to fulfill several requirements. A structure resolution down to <100 µm is necessary in order to print electrode structures according to the underlying microfluidic structure. Silver nanoparticle based conducting structures are not allowed if the electrodes are in contact with the fluid due to the potential genotoxic and cytotoxic properties of silver nanoparticles, thus other, biological inert conducting materials such as gold have to be used. Finally, under the influence of the electric field the electrodes have to withstand electrolysis. Fig. 5 shows an example for structure resolution where electrodes have been printed onto a polymeric PET substrate.

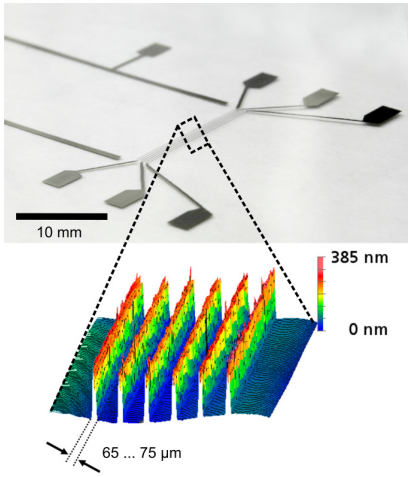

***Figure 5**. Inkjet-printed electrode layout for microfluidic applications, photograph (upper) and profile (lower).*

Chemosensors for pH Detection

Sensing of process parameters for a µFFIEF in the separation chamber is normally performed indirectly or by subsequent offline analytical methods. This can be overcome by an integration of a fluorescent pH sensor layer into an FFE microfluidic chip, monitoring isoelectric points directly [3]. For a fast response time inkjet printed micron-sized pH sensor structures are suitable and can be manufactured by printing a solution of FITC-dextran nanoparticles in polyhydroxyethylmethacrylate. Fig. 6 shows on the left inkjet printed 320 µm spherical spots on glass; the pH sensitivity was calibrated with Britton Robinson buffers and resulted in a pK_a of 7.1 at the ionic strength of the ampholyte mixture used for IEF (Fig. 6, right). Response times t_{95} of 0.8 s to 4.3 s could be achieved by these printed structures.

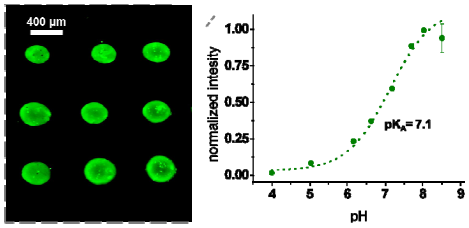

***Figure 6**. Microscopic image of pH sensor array (left) and the obtained pH calibration curve (right)*

For an example of using this pH chemosensor the separation of biomolecules was examined. Therefore proteins were labeled with a red fluorescent P503 dye that does not alter their charge. IEF was performed in a microfluidic chip according to Fig. 7 with protein solutions injected via a central fluidic inlet, flanked by channels containing 1 % ampholyte pH 3 -10, and acidic and alkaline sheath flows. An electrical current was now applied via discrete copper electrodes attached to the electrolyte channels containing acidic/ alkaline buffer, so a pH gradient could be established in the separation chamber and be monitored via the green fluorescence of the pH sensor (Fig. 8, right). Proteins and other biomolecules were separated by IEF and monitored in the red channel (Fig. 8, left).

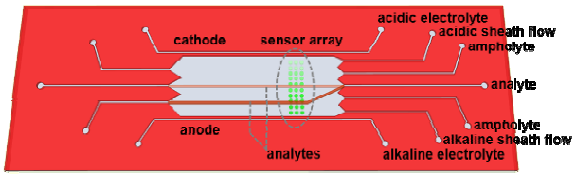

***Figure 7**. Schematic of a µFFE chip with an integrated pH sensor array*

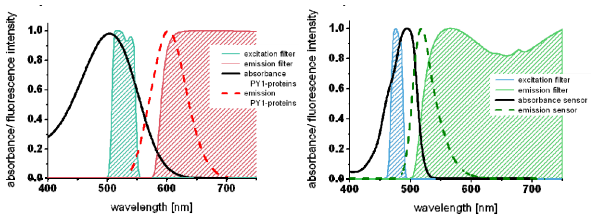

***Figure 8**. Spectral channels used for protein (left) and pH sensor (right) monitoring, respectively*

The channel overlay allows a visualization of the pH gradient and the protein fluorescence and therefore online pH quantification of analytes that are focused electrophoretically via IEF (Fig. 9).

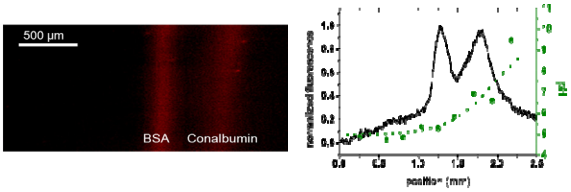

***Figure 9**. Separation of the proteins BSA and conalbumin by µFFIEF (left) and protein fluorescence in the separation chamber (black) with online monitored pH by the integrated sensor structure (green)*

Pumps for Fluid Distribution

Finally, the fluids and reagents under investigation in a LoC need to be distributed through the channel, which in automated systems is done by pumps and valves. An all inkjet printed membrane pump actuator can [4] be configured as a bimorph setup that consists of an Electro Active Polymer (EAP) layer sandwiched between two silver electrodes on a passive substrate (Fig. 10).For manufacturing first samples of such a membrane pump, first the bottom electrode was printed using commercially available silver nanoparticle ink from Harima Chemicals Inc. (NPS-JL) and sintered using a low-pressure argon plasma exposure. The argon plasma enables a sintering of the metal nanoparticles without damaging temperature-sensitive substrates. On top of the bottom electrode, the EAP layer was printed using a solution of piezoelectric co-polymers from Solvay Solexis S.p.A. (solvene™). Several layers were printed to reach a total thickness of 10 µm to 15 µm. After printing, the polymer layer was tempered at 130 °C. On top of the EAP, a top electrode layer then was printed and sintered using again argon plasma sintering. The EAP exhibits piezoelectric behavior when poled electrically. A voltage that is applied to the electrodes thus leads to piezoelectric strain in the EAP layer, in combination with the passive substrate this leads to bending. Fig. 11 (left) shows a circular membrane EAP actuator on a ring-shaped metallic holder for demonstration. In that configuration the membrane deflection (Fig. 11, right) leads to a volume change under the deflected membrane. On top of pumping chamber with inlet and outlet valves this structure realizes a pumping function.

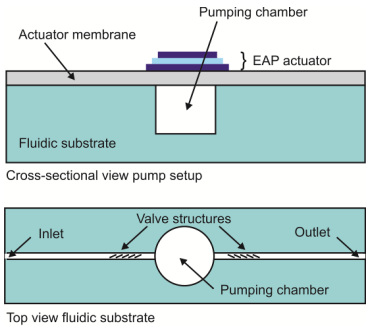

***Figure 10**. Principle of a micro pump with printed EAP membrane*

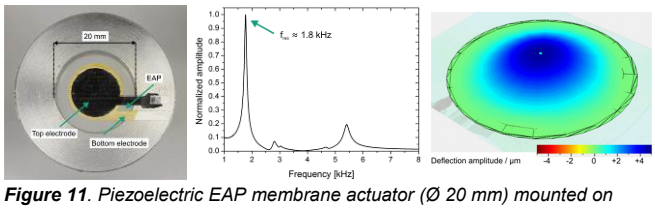

***Figure 11**. Piezoelectric EAP membrane actuator (Ø 20 mm) mounted on metallic ring (left), first resonance frequency of ca. 1.8 kHz (center) and vibrometer measured,110 V driven deflection at resonance of about4 µm (right)*

For the application of the membrane actuator in a micro pump it is necessary to generate a large volume changes in order to achieve reasonable pump rates. At the same time, for LoC devices it is desired to apply only moderate driving voltages. Static driving of EAP actuators typically requires voltages between several hundred volts and the kV range. In contrast, driving the membrane actuator at the resonance frequency high deflections at moderate driving voltages can be obtained. A laser scanning vibrometer was used to characterize the frequency-dependent deflection amplitude (Fig. 11, center and right). The first resonance frequency was measured to be ca. 1.8 kHz for a membrane diameter of 20 mm. The maximum deflection amplitude of about 4.5 µm occurs in the membrane center, resulting in a volume change of ca. 0.5 µl for a half-stroke of the membrane actuator. Based on this the pumping rate for the application of such actuators in a micro pump can be estimated. When a valve efficiency of 1% is assumed, the predicted pump rate is ca. 500 µl/min. This rate is in an order of magnitude that is suitable for LoC devices.

Outlook: A Complex Lab-on-Chip System

One possible application of enhanced functionality for a LoC device is the already mentioned polymerase chain reaction (PCR) that is widely used for the amplification of DNA sequences in order to search e.g. for salmonella in food or for specific antibodies in the human blood to make the use of drugs more safe [5]. Besides probe preparation the DNA amplification by multiple temperature cycles at two or more different temperature plateaus is the basic process of a PCR and is realized either in single chambers undergoing different temperature leveling or in continuous flow where the fluid containing the DNA sequences to be amplified repeatedly flows through zones at the desired temperature level. Fig. 12 shows a LoC amplification system designed for a continuous flow PCR, containing four different heating zones for temperature leveling, one fluidic channel that crosses all the different temperature zones and at both ends of the channel a chip-integrated micro pump that enables for driving the fluid back and forth in repeated cycles through the temperature zones. Such a LoC at the size of a microscope slide exhibits the needs as well as the possibilities to integrate functionalities on the chip.

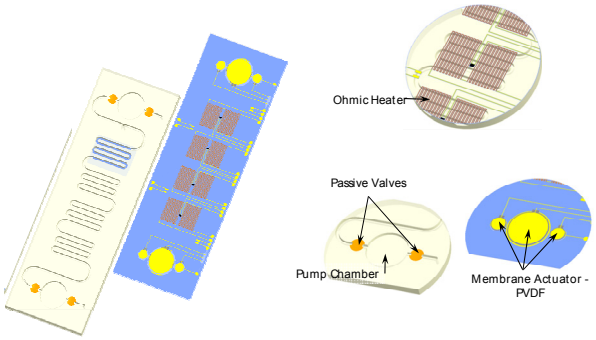

***Figure 12**. A fully chip-integrated PCR LoC, view without and with cover foil on the left, details of the chip integrated heaters and micro pumps on the right*

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Author Biography

Erik Beckert received his Diploma (1997) and his PhD (2005) in mechanical engineering from Technische Universität Ilmenau, Germany. Since 2001 he is working at Fraunhofer Institute for Applied Optics and Precision Engineering (IOF) in Jena, Germany. His work also focuses on leading the institute’s activities about printing of functional materials.

