Optofluidic Microsystems for Application in Biotechnology and Life Sciences

S. Sinzinger, B.P. Cahill, J. Metze, and M. Hoffmann

Abstract Microfluidic systems have been shown to have a large potential for applications in biotechnology and life sciences. This includes traditional fluid actuators alongside alternative techniques such as nanofluidic and multiphase systems. While the fluidic systems are often highly miniaturized and integrated, the systems for optical testing, probing, and manipulation lack this degree of integration. The goal of our research focuses on the integration of fluidic, optical, and mechanical functionalities in complex optical microsystems. We report on fundamental investigations carried out in close cooperation between the Institute of Micro- and Nanotechnologies (IMN-MacroNano®) at Ilmenau University of Technology, and the Institute for Bioprocessing and Analytical Measurement Techniques (IBA), Heiligenstadt, Germany.

Keywords Microfluidics • MOEMs • Optofluidics • Optical tweezers • Optical Microsystems

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Contents

1 Introduction

Intensive efforts to use microsystems for performing biotechnological tasks have been undertaken since the 1990s. The keystone of the necessary technical foundations for such systems is microfluidics, the technological basis of which requires the integration of mechanics, optics, electronics, and also nanotechnology. The great potential for exploitation of this approach in the field of biotechnology and life sciences is shown by the explosion of the number of publications in this field by many different working groups worldwide.

In the field of biotechnology and life sciences, cells or biological specimen are cultivated as well as manipulated and analyzed. Optical procedures for micromanipulation (so-called optical tweezers) and analytics play a leading role in cell culture and biotechnology. In this respect, the possibilities for reproducible contactless manipulation of cells or droplets that contain cells of comparable orders of magnitude could be of use. In particular, for cells in contact with interfaces, for example, micromechanical manipulators or phase boundaries can lead to undesired interactions or even completely changed cell characteristics.

While substantial research and development activity has already been expended on developing dispensing technology to its present state, additional opportunities for development still exist in pipetting and transportation processes for biogenic media and miniaturized biotechnology. This includes traditional fluid actuators alongside alternative techniques such as nanofluidic and multiphase systems. While such fluidic systems are often highly miniaturized and integrated, the systems for optical testing, probing, and manipulation lack this degree of integration. The goal of our research focuses on the integration of fluidic, optical, and mechanical functionalities in complex optical microsystems. We report on fundamental investigations carried out in close cooperation between the Institute of Micro- and Nanotechnologies (IMN-MacroNano®) at Ilmenau University of Technology, and the Institute for Bioprocessing and Analytical Measurement Techniques (IBA), Heiligenstadt, Germany.

2 Microsystems for Biotechnology and Life Sciences

The micromanipulation of cells is a research field that requires a great deal of innovation; in particular, it is necessary to deal with the current awareness in cell biology that making mechanical contact with a cell is likely to affect its further development. The capture and directed transport of cells by means of "light" or "electric fields" could be a way to address this challenge. Therefore, it is the more important to open new research directions in optofluidic microsystems. The hitherto accepted methods for removing cells have been by taking a full sample and/or by using a bypass with an integrated three-way valve that facilitates sample removal. By working with integrated optofluidic microsystems, cultivation in bioreactor volume sizes from milliliter (mL) down to microliter (μL) range is permitted. Coupled with the optical techniques for micromanipulation presented here, this represents an important cost reduction. At the same time, the possibility of parallel processing is strongly improved by the considerably smaller space requirement.

The authors have carried out comprehensive investigations for screening of applications with regard to the sensory and handling potential of the system. The influence of the surface properties and various coating materials on the performance of microfluidic channels was fundamentally examined. In particular, the control of the adhesion of proteins and microorganisms to channel walls is of great practical significance.

Optical tweezers have become an established handling tool in cell technology in recent years. Although hitherto developed systems have been characterized by their macroscopic experimental setups, completely new applications are opened up by microengineered and integrated systems.

From a biotechnological point of view, possible applications in microsystems are the following:

1. Holding and/or directing cells.
2. Controlling the distance between cells through start–stop laser sequencing.
3. Diversion of cells from a channel can be achieved by bringing the cell to the intersection point of two fluidic streams and switching the flow direction.
4. Defined positioning of cells on a sensor.
5. Measurement of adhesion forces through investigation of the interaction of the cell and other object.
6. Cell fusion through application of two laser tweezers or one laser tweezers and an electric field cage.

One could imagine handling transparent fluid compartments in the same way. These aqueous compartments embedded in a nonpolar medium, for example, could serve as a reactor for microorganisms.

To this end, the Department of Bioprocess Technology of the IBA has developed a technology platform that in the meantime led to the phrase "pipe-based bioreactors" being registered as a trademark (Fig. 1).

Fig. 1 "Pipe-based bioreactors" is a registered trademark of iba

The principles of flow injection analysis systems were published a few decades ago [1, 2]. This enabled the automation of assays in a fluidic phase in combination with low consumption of reagents and sample liquid. In recent years, a strong positive trend in the development and application of microfluidics is ascertainably reflected in an enormous increase of patents in this field and a remarkable number of companies which produce and commercialize miniaturized analytical devices [3]. Custom-made microfluidic chips and entire measuring systems are provided by several companies, and microfluidic systems have been adapted to most of the common analytical techniques [4]. For many applications, the theoretical fundamentals even on the nanoliter scale have been studied and implemented [5]. Widely used fabrication materials are silicon, glass, and a variety of polymers [6]. By minimizing the dimensions of fluidic structures, the surface-to-volume ratio increases in a way that surface interactions become almost dominant. Therefore, the surface properties of microfluidic channels are crucial to the behavior of the liquids, especially in a two-fluid system. Today, the microtiter plate consisting of a flat plate with multiple open wells at a microliter scale is still standard in analytical research and clinical diagnostic testing. Further miniaturization of assay systems requires the use of closed fluidic systems to prevent vaporization, a serious problem during handling of very small sample volumes. Downscaling intensifies this effect, and the evaporation of such droplets occurs very quickly and rapidly. To prevent this phenomenon, it is evident that test formats have to be established in closed microfluidic systems. One of the main features will be the use of many separate fluidic segments to enable self-contained experimental conditions in each droplet. The utilization of flow channels with small diameters requires a precise control of the flow conditions regarding fluid transport and mixing conditions caused by dispersion and diffusion. Working with segmented flow in closed microchannels, which is characterized by fluid volumes separated by air or immiscible carrier liquids, leads to distinct compartments in the system. Such droplet-based microfluidic platforms allow microfluidic unit operations, whereby lateral diffusion (Taylor diffusion) is prevented [7]. In addition to properly designed geometries of the flow channels, surfaces which hinder wetting by the droplet fluids are required. Otherwise, droplets will partially stick to the walls and may lose their integrity. The droplets can be generated at high reproducibility and high frequency at nanoliter volumes by the use of specially designed microfluidic chips [8–10]. The channel walls composed of glass [8] or polydimethylsiloxane (PDMS) [9] are coated with octadecyltrichlorosilane (ODTS), resulting in a hydrophobic surface. Silane molecules form a covalent binding to hydroxyl surface groups and can also covalently link via condensation to neighboring silane molecules, resulting in an ordered monomolecular film coating [11]. The alkyl chains are oriented toward the liquid

phase and determine the hydrophobicity due to their molecular structure. Alkylsilane bindings are found to be very stable against chemical and thermal factors [12]. Therefore, wetting of the walls by aqueous droplets was minimized, and the droplets embedded in an oily carrier liquid take a round convex shape. Due to these characteristics, the droplets maintain their regular distances and can be transported serially through the system like pearls on a string. Recently, microorganisms from soil samples have been cultivated in media droplets by incubation in polytetrafluoroethylene (PTFE) capillaries [13]. Growth rates similar to that of shake flask cultures could be observed even in the case of strictly aerobic bacteria [14].

A material of great current interest for manufacturing bio-micro-electro-mechanical systems (MEMS) is the epoxy-based UV-sensitive polymer SU-8. Examples of microchannels fabricated using this polymer have recently been published [15–17]. It provides biocompatibility [18] and chemical inertness against most biological substances [19] and, due to its transparency, allows optical inspection during system operation. The low surface roughness and vertical sidewalls enable the use of different optical analytical techniques (e.g., fluorescence-based or spectrometric assays). Furthermore, it facilitates low-cost batch processing and is suitable to form deep and smooth fluidic channels in the necessary geometrical dimensions for segmented flow operation [20]. The possibility of easily integrating functional components (electrodes, waveguides) in intermediate layers and the low temperature bonding capability make it a suitable material for the manufacturing of hybrid biofluidic MEMS.

3 Scientific Results

The stability of the silane monolayer coated on the surfaces of the channel of the microfluidic chip built completely from glass to infrared light exposure has been investigated. Experiments with an optical tweezers laser setup showed that yeast cells could be held in the middle of the microchannel. These experiments were also confirmed for aqueous compartments in a nonpolar carrier medium. Yeast cells could be captured and held in the focus of the laser tweezers while a compartment moved through the channel. A change of direction in throughflow could not be observed. Corresponding experiments with structured illumination showed that cells were pushed out of focus and moved further in the direction of flow. Furthermore, fluidic characterization and various experiments to optimize the fluidic properties of prototype microfluidic chips as well as chips with SU-8 channels and others with integrated electric field cages were carried out. The current results show great promise (Fig. 2).

The application of segmented flow technology requires a hydrophobic surface, and in many cases, this requires a robust permanent hydrophobization method for channel walls (Fig. 2).

A special chip holder was built for the electric field cage experiments (Fig. 3) so that the chip could be connected electrically and fluidically.

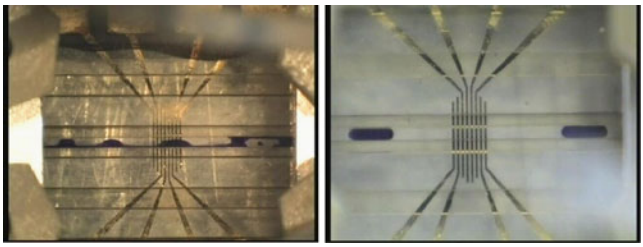

Fig. 2 Fluidic characterization of the SU-8-chip channel (aqueous phase (*blue*), nonpolar carrier fluid (*colorless*)): *left*: the aqueous phase adheres to the walls in segmented flow on untreated SU-8 channel walls; *right*: after chemical modification: flawless passage of compartments through the channel

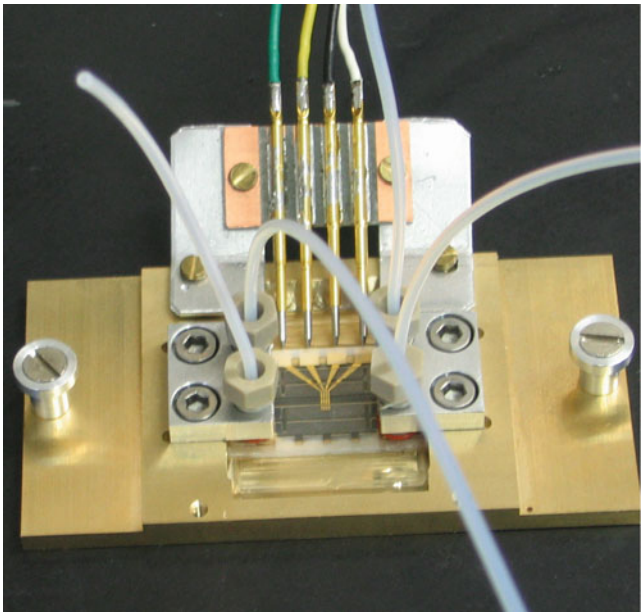

Fig. 3 Spring loaded electrical contacts connecting the optofluidic microsystem with SU-8 channels

Experiments were carried out to investigate the influence of dielectropohoresis on yeast cells, the results of which were extremely positive. Yeast cells could be transported in the channel and gathered at electrodes under the influence of high-frequency alternating current fields (Fig. 4).

One of the interesting results of the experiments was the improvement of the effectiveness of planar optical systems through integration of (discrete) refractive elements. It was shown that sustainable progress can be achieved through the combination of mechanics, optics, electronics, and nanotechnology in the field of micro-opto-electro-mechanical systems (MOEMS) [21]. The simultaneous

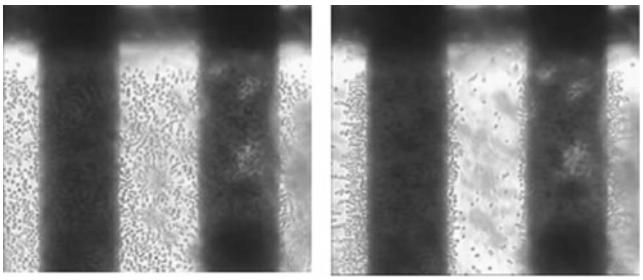

Fig. 4 Dielectrophoresis of yeast cell suspensions in a microfluidic channel (gold electrodes appear black). *Left*: no electric field applied, *right*: high-frequency alternating current fields applied

integration of fluidic systems allowed the suitability of such systems for cell cultures to be tested. In connection with this point, it is important to note that microtechnology enables the consumption of culture medium, which can be highly expensive in biotechnology, to be minimized.

4 Technologies for Optofluidic MOEMS

Special care has to be taken to design optofluidic chips that fulfill various different requirements: a fluidic part that guarantees the smooth transport of fluid segments, and an optical layout that is suitable for optical functionalities and full biocompatibility of the system. The additional integration of electrodes requires an additional process that allows one to include fully coated electrodes beneath a thin isolation. So the required properties of the optofluidic chip are as follows:

Optical requirements

- High transparency/very low absorption, under intensive laser radiation at a wavelength of $\lambda = 1{,}064$ nm
- High surface quality (flatness, roughness)
- Refractive index close to that of the fluid, no major index steps

Fluidic requirements

- Lateral resolution of 5 μm or better at a channel height larger than 200 μm
- Reliable coating with hydrophobic layers
- Very low surface roughness (stick/slip of fluidic segments)

Biological and chemical needs

- Compatibility with the carrier fluids of segmented flows (chemically inert against, e.g., alcohols, DMSO, and tetradecane)
- Biocompatibility of all parts and materials
- Temperature resistant to at least 40°C

– Transparent in the wavelength range $450 < \lambda < 600$ nm (fluorescence, optical inspection)

General issues

– Low-cost processing of devices, favorably in a batch process
– Easy change of layouts by changing only masks (requires design rules)

A careful analysis of these requirements led to the selection of SU-8-based microchannels. SU-8 is highly transparent in the required wavelength regions, and it can be fabricated in thicknesses from some microns up to some hundred microns. The device layout is achieved by simple UV lithography and thus changes in the chip design only require a new lithographic mask. This is an important advantage for a rapid prototyping against, e.g., injection molded devices that require much more complex processes for mold preparation. Nevertheless, a fabrication of larger quantities of these devices by injection molding is a suitable option. Optofluidic devices made from wet-etched or milled glass require a careful surface treatment after etching to achieve optically flat surfaces that are suitable for the optical part.

Silicon-based devices suffer from the lack of transparency in the required wavelength range. Even at 1,064 nm wavelength, silicon has a significant absorption resulting in a localized heating of the walls and the fluid close to the laser beam.

Additionally, the layer-by-layer approach of stacked SU-8 layers allows the integration of electrodes with or without contact to the liquid in the flow channels. SU-8-based chips are a perfect solution for fast sample preparation and tests.

5 Layout of Microfluidic Channels for Segmented Flow Applications

The microfluidic channels for this application should have a height of 200 μm, which is rather high for typical MEMS processes. On the contrary, "smooth" shapes are required to prevent the droplets from sticking to curved areas or at the inlet and outlet port. Also, a reliable closure of the channels is needed, which in itself does not cause sticking areas for the fluid. For all these reasons, an SU-8-based bonding process was chosen as the most suitable process. Both halves of the fluidic chip can be prepared in a very similar way; the key issue is the bonding process.

As the additional investigation of dielectrophoresis also requires capacitive electrodes reliably isolated from the fluid, a process was investigated that allows a full protection of the electrodes.

6 How to Fabricate Closed Channels Made From SU-8?

SU-8 is a UV-curing polymer suitable for spin coating. It can be applied in various thicknesses depending on the viscosity of the liquid polymer. In a final curing step after UV exposure and development, it can be fully cross-linked. After this, the polymer is extremely stable; even oxygen-based plasma etching does not remove the material significantly. The adhesion of SU-8 to typical substrate materials is usually good, although the stress within the polymer has to be taken into account to avoid stress-induced damage. Therefore, a proper curing regime is very important (Fig. 5).

As a substrate for both parts of the fluidic device (upper and lower half of the channel), commercially available borosilicate glass wafers were chosen. It was found that a prime coating of the glass surface with a thin SU-8 layer improves the adhesion of the SU-8-based parts against samples without closed film. The advantage of thin SU-8 films is that all the surfaces of the channel that the fluid contacts are from SU-8. For film preparation, low-viscosity SU-8 resulting in a 4–5-µm-thick film during spin coating was selected.

This thickness is also high enough, to bury electrodes (about 100-nm thick) completely that are favorably fabricated on the glass. The electrodes themselves are made from chromium (adhesion promoter on glass) and gold, and are structured by lift-off.

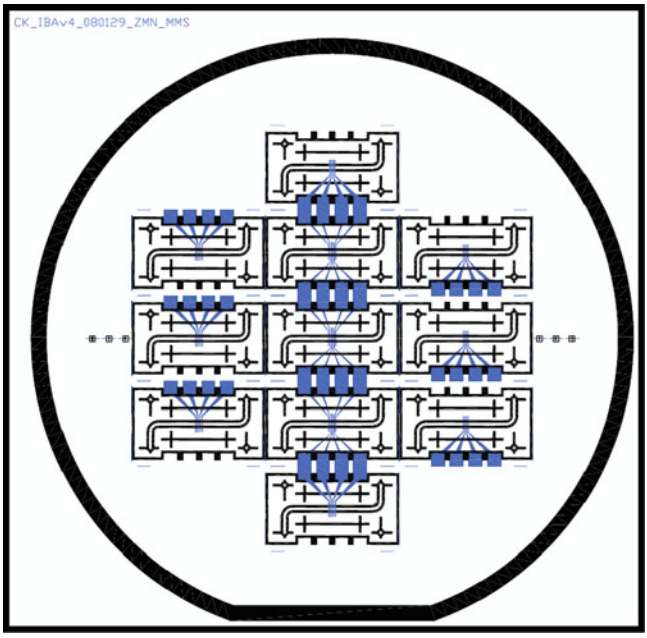

Fig. 5 Layout of test chips with a 2-mask process; wide *black lines* show the remaining SU-8 walls; electrodes with contact pads are also shown (*gray areas*)

During process development, it was found that large areas of thick SU-8 films that remain on the substrate tend to show cracks after cross-linking. Intrinsic stress on these large areas leads to a significant damage. Also, an insufficient drying after spin coating caused problems due to bubbles that appeared in the cross-linked polymer. As a result of these findings, a channel geometry was defined that prepares walls along the channels, only, to reduce the area of closed thick SU-8 layers. As a rough estimation, the wall thickness was chosen in the range of the channel width, and all structures exhibit nearly the same width, including supporting posts and walls that stabilize the sandwich structure.

One of the most important properties of SU-8 that is used here is the stepwise lithography: It is possible to expose areas of SU-8 to UV followed by a bake to support a soft cross-linking. In a next step, another layer of SU-8 can be applied and again an exposure can take place. In this case, the already performed bake makes the bottom layer insensitive to further UV radiation. After a second bake, the unexposed SU-8 of both layers can be washed out with a suitable developer; only the structures exposed to UV directly after spin coating remain. If the baking procedures are performed with great care, the structures are now stable enough to dice and handle the chips, but the cross-linking is not yet finished.

After careful cleaning and inspection of the separated chips, the upper and lower parts are aligned against each other and then the full stack is baked again, now at elevated temperature. During this procedure, a tight connection between the two SU-8 surfaces appears. No further layers of SU-8 or other glues are required. This is a big advantage for the use in biological systems because it avoids any type of glue or monomers that could come into contact with the inner walls of the channel. The only critical step is the bonding because it is responsible for the smoothness of the fluidic walls.

The use of otherwise functional materials used in MOEMS also for bonding is in general a very suitable technology. As most of these materials are already tested to be biocompatible, it avoids the addition of any other glue that may cause problems due to residual monomers. In this case, the most critical requirement is a transparent cover plate on an Si-based microfluidic chip. In principle, this technology is also suitable for glass–glass bonds.

For tight interfaces between polymers and silicon, a glueless technology is highly suitable: Stubenrauch et al. [22] used deep reactive ion etching (DRIE) to produce needle-like structures around through-holes in microfluidic devices in silicon. The needles are etched in a way making them very robust. A lithographic step for each needle is not required. Leopold et al. [23] investigated the process in detail and showed that carbon-based chains are the origin of a self-masking within the first cycles of etching. But how to join a polymer-based part to the silicon needles?—At least all thermoplastic polymers can be easily assembled. At first, the chip is heated above T_G of the polymer. If the polymer is now brought into contact with the device, the needles melt the polymer and enter the device. After cooling down, the polymer has a very tight contact with the needles. Again, no glue is required. This process is currently limited to silicon–polymer interfaces due to the material properties of silicon (Fig. 6).

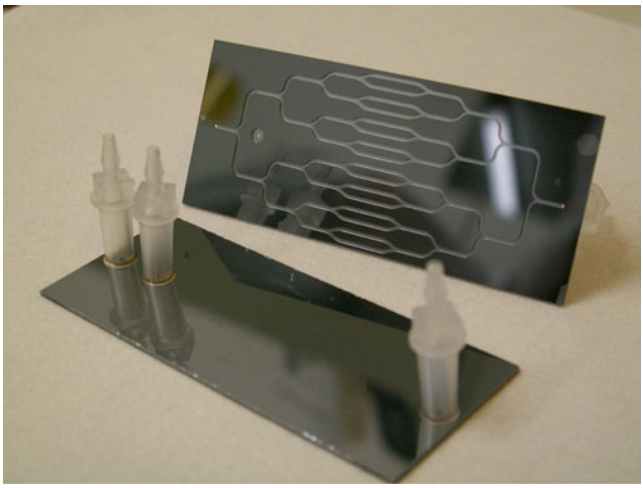

Fig. 6 Si-based fluidic chip with glueless assembly of connectors based on Si-glass

7 Coating of the Fluidic Channels

After chip preparation, the surface of SU-8 has to be prepared with hydrophobic coating [20]. It has to prevent sticking of fluid segments to hydrophilic surface elements, especially at inhomogeneities of the channel walls.

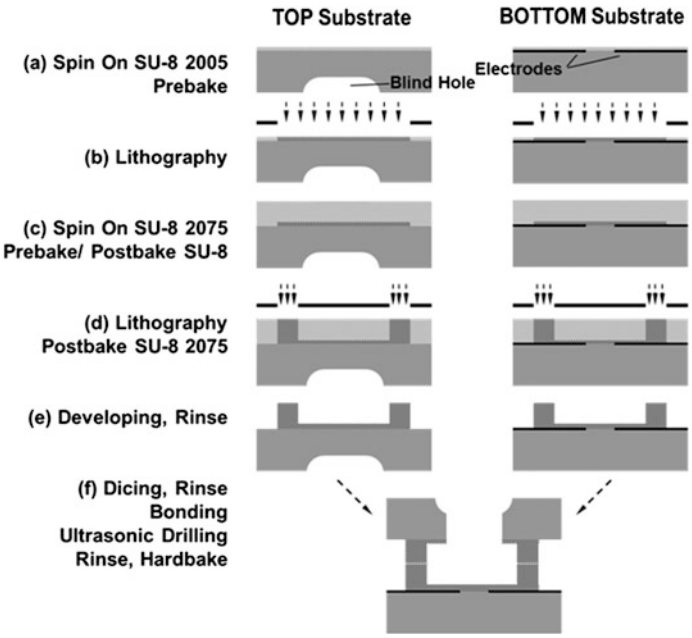

Fig. 7 Process flow for the fabrication of SU-8-based microfluidic devices with buried electrodes

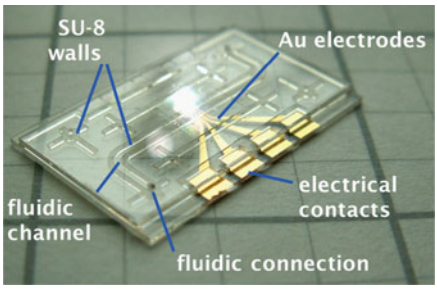

Fig. 8 Micro-opto-electro-fluidic chip based on SU-8

For sufficient adhesion of the silane layer, an activation of the surface is required. Here, the extremely high stability of SU-8 against oxidizing agents is helpful. The channels are flushed with hot piranha solution (a mixture of sulfuric acid and hydrogen peroxide) for some minutes. Surprisingly, this very aggressive solution does not attack the organic SU-8 resin; it only changes the surface in a way that the following coating has very high adhesion.

Careful purging between the steps and after silanization is required because of the risk of cross-contaminations. Here, the absence of capillary voids due to misalignments or design-induced errors during lithography is essential (Figs. 7 and 8).

8 Optical Microsystems for Micromanipulation and Sensing

A variety of approaches to microoptical systems integration have been suggested and demonstrated for applications such as optical interconnects or optical sensing [24]. Among these, the planar optical integration of free-space optical systems enables the most compact and stable integration of optical systems. To this end, the optical axis of the system is folded in such a way that all optical components can be arranged in a planar configuration [25]. Planar fabrication processes, in this case, can be exploited not only for the fabrication of the individual components. The whole system can rather be fabricated with one single fabrication procedure (lithographic or non-lithographic), where all components are, e.g., integrated in the surfaces of a single transparent substrate [26, 27]. Due to the folded optical axis, very specific design knowledge and fabrication technologies are necessary to avoid aberrations and achieve systems with optimum performance [28, 29]. These fundamental concepts for layout, design, and fabrication of planar optical systems can be exploited for the realization of complex optofluidic systems for micromanipulation and sensing in microfluidic environments [30–32]. Specifically, innovative fabrication technologies such as ultraprecision micromilling combined with diffractive microstructures offer new potential for the integration of complex optofluidic functionality [33, 34].

The fundamental concept of optical tweezing is based on the forces resulting from the interaction of light with microscopic particles. As first noted by Ashkin in the 1970s and 1980s [35], in addition to the repulsive scattering forces, the refraction and scattering of photons at microscopic particles may also result in gradient forces, allowing a 3D trapping of small particles such as cells or bacteria [36]. One of the most prominent challenges for optical tweezing in microfluidic environments stems from the limited working distance (i.e., distance between the objective lens and the optical trap) of conventional optical tweezing systems. In order to achieve reasonable trapping forces (i.e., reasonably stiff traps), it is necessary to use highly focused (high NA) beams for trapping. In the generally applied inverted microscopes, this automatically results in extremely small working distances in the range of 100 μm in immersion liquids. This contradicts the application in microfluidic channel systems with channel walls in the range of several 100 μm. Such an environment imposes specific challenges to the integration of the optical functionality.

In order to achieve optical manipulation within the segmented flow systems, we applied two different strategies. On the one hand, the concept of counter-propagating traps [37–39] has been demonstrated to work within the microfluidic systems made of transparent substrates such as fused silica or SU-8. For a second approach, we demonstrated the integration of microoptical elements into the microfluidic system in order to enhance and adapt the optical performance to the optical microsystem [40].

For counter-propagating optical tweezers, the fluidic channel is illuminated from opposite directions by moderately focused laser beams. Due to the two beams propagating in opposite directions, the scattering forces on the microparticles are compensated in such a way that 3D trapping is possible even at relatively large distances of several millimeters through the channel walls with low NA beams. Figure 9a schematically shows the layout of the system. Two microscope objective lenses optimized for large working distances are used to focus the laser beam from

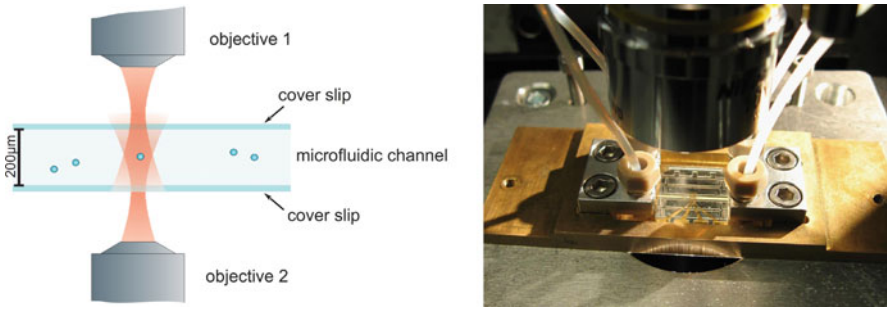

Fig. 9 (**a**) Schematic configuration of counter-propagating optical tweezers; (**b**) close-up of the experimental setup showing the upper objective lens and the microfluidic system including connectors

opposite directions into the fluidic channel. The radiation pressure and thus the positioning in axial direction can be compensated by controlling the focusing as well as the laser power in the two counter-propagating beams. At the same time, the lateral forces can be used to trap and manipulate the particles perpendicular to the optical axis. Figure 9b shows that this approach allows one to combine the optical trapping setup with the microfluidic system requiring significant space for, e.g., the fluidic connectors. We successfully demonstrated the trapping and manipulation of yeast cells within segmented flow systems with this configuration.

Microsystems integration allows one to integrate optical functionality directly into the microfluidic system. This enables the integration of additional optical functionality such as diffractive optical beam splitters. Furthermore, it is possible to define precisely the interface between the optical environment and the fluidic system. Figure 10 shows the integration of a beam-splitting diffractive optical element (DOE) into a microfluidic channel system replicated in PDMS. By integrating the DOE into a converging wave front (Fig. 10a), it is possible to achieve a compact configuration which is easy to integrate in an optofluidic setup, as shown in Fig. 10b. The DOE in such a configuration may serve a variety of purposes. The most widespread application is the generation of multiple optical traps. In this case, the multiple diffraction orders generated by the DOE may be used as individual traps. In the case of the use of immersion lenses, specific care has to be taken in order to guarantee the necessary depth of the phase profile of the DOE. In the example shown in Fig. 10, this has been achieved by additionally covering the DOE with a cover slip in order to avoid index matching of the immersion oil and the DOE profile. Figure 11 shows a sequence of photographs illustrating the performance of the system with an array of diffraction orders generated inside the fluidic channel. Each of the large NA diffraction orders may be used for the trapping of individual particles in the fluidic flow. Alternatively, the diffractive optical elements may be applied for the optimization of the optical performance of the trapping system. This way it is possible to correct for aberrations in the focus distribution that occur due to the fluidic environment. By integrating an additional focusing functionality, the DOE may also be used to extend the working distance between the external optical elements and the integrated optofluidic system.

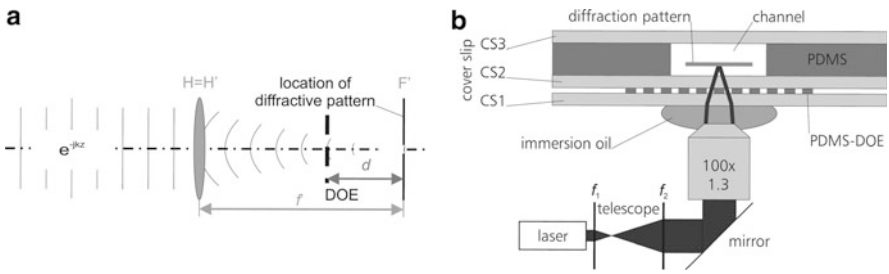

Fig. 10 Optofluidic integration of diffractive beam splitting in a compact microfluidic system: (**a**) schematic of the optical setup for a compact and flexible integration of the DOE into the optical trapping system; (**b**) schematic of the integrated system

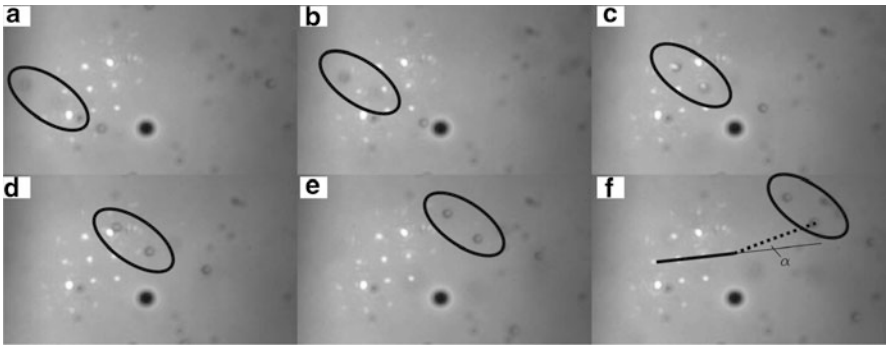

Fig. 11 Sequence of photographs showing 3D optical trapping inside an optofluidic system containing an integrated diffractive optical beam splitter

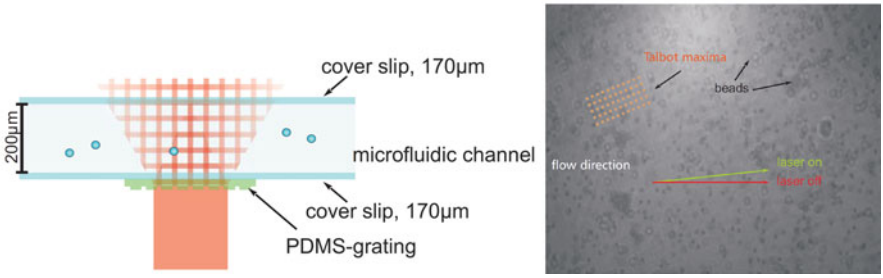

Fig. 12 Demonstration of an integrated multiple trapping system based on the Talbot effect: (**a**) schematic of the integrated system; (**b**) photograph of the particle flow inside the microfluidic channel showing the particle deflection due Talbot carpets

A third and intriguingly simple optical configuration for the implementation and integration of compact optical trapping systems is based on the so-called Talbot self-imaging of periodic diffraction gratings [41, 42]. Due to diffraction and interference of the light in the area behind a diffraction grating, a three-dimensional periodic pattern of light intensity is formed. This light distribution is sometimes called a Talbot carpet. Due to the fact that the formation of such Talbot carpets occurs without any focusing elements such as lenses or objectives, they provide the conceptually simplest approach for the formation of multiple optical traps. For the integration, it is sufficient to integrate a periodic diffraction grating into the fluidic system (Fig. 12). Upon illumination by a plane wavefront, the periodic Talbot carpets are formed. These provide a large array of multiple optical traps. Figure 12b shows the deflection of particles in a fluidic flow through such a periodic array of Talbot traps. The Talbot carpet cannot be observed here since the IR wavelength of the traping laser is filtered in order to be able to observe the flowing particles.

Since the deflection of the particles, in this case, depends on the size and further characteristics of the particles, such a highly compact system can be applied, e.g., for particle sorting inside the fluidic system.

These examples demonstrate the potential of microoptical technology to improve the applicability of optical tweezing in microfluidic systems. The combination and integration of microoptical components help to define and improve the control of the interface between the inverted microscope used to provide the highly focused trapping beam and the microfluidic system. Further improvements can be achieved if the whole optical system is designed according to the very specific needs of optical trapping. In this case, the efficiency and the working distance can be improved significantly [43].

9 Conclusions and Future Perspective

The application of a laser tweezers to hold yeast cells in the middle of a channel showed that the cells could be retained in both segmented and continuous flow. Yeast cells could be trapped and held in the laser focus during the passage of the compartments through the channel.

In order to achieve this for both the chip with SU-8 channels and the chip with integrated electric field cages, it was necessary to carry out various fluidic characterizations to improve the fluidic properties of the prototype microfluidic chips (see Fig. 13).

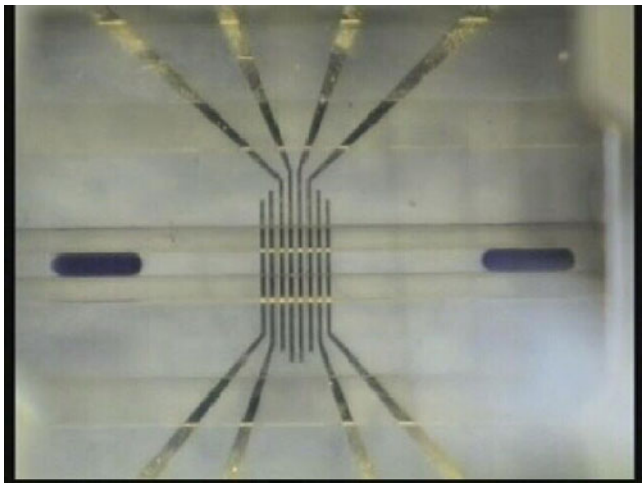

Fig. 13 Passage of compartments through the SU-8 Chip channel with integrated electric field cage electrodes

It can be expected that new concepts that make use of the complexity of integrated microsystems will lead to new applications in biotechnology and cell technology.

These concepts contain a large economic potential, as the possible functionality of the analysis platforms is substantially increased and the handling is decisively simplified. At the same time, microtechnological integration allows the consumption of very expensive culture media common in biotechnology to be minimized.

The development of completely integrated microsystems with complex optical functionality on the basis of planar optical integrated systems offers a very wide spectrum of applications across cell technology and biotechnology. Solely on the basis of the efficient handling of these systems in comparison with classic modular setups, many opportunities are opened in the already existing markets of, for instance, sensors, communications, and safety technology. On the contrary, complex microsystems open new areas of application in fields that were previously excluded by the instability and bulkiness of classically built systems. For these reasons, an economic breakthrough is predicted for integrated optical microsystems in the next years.

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References

1. Ruzicka J, Hansen EH (1981) Flow injection analysis, chemical analysis. vol 62. Wiley, New York
2. Valcarcel M, Luque de Castro MD (1987) Flow-injection analysis. Ellis Horwood, Chichester
3. Haber C (2006) Microfluidics in commercial applications; an industry perspective. Lab Chip 6:1118–21
4. Dittrich PS, Manz A (2006) Lab-on-a-chip: microfluidics in drug discovery. Nat Rev Drug Discov 5:210–8
5. Squires TM, Quake SR (2005) Microfluidics: fluid physics at the nanoliter scale. Rev Mod Phys 77:977–1026
6. Niemeyer CM, Mirkin CA (2004) Nanobiotechnology. Wiley-VCH, Weinheim
7. Haeberle S, Zengerle R (2007) Microfluidic platforms for lab-on-a-chip applications. Lab Chip 7:1094–110
8. Henkel T, Bermig T, Kielpinski M, Grodrian A, Metze J, Köhler JM (2004) Chip modules for generation and manipulation of fluid segments for micro serial flow processes. Chem Eng J 101:439–45
9. Adzima BJ, Velankar SS (2006) Pressure drops for droplet flows in microfluidic channels. J Micromech Microeng 16:1504–10

10. Song H, Tice HD, Ismagilov RF (2003) A microfluidic system for controlling reaction networks in time. Angew Chem 42:767–72
11. Allara DL, Parikh AN, Rondelez F (1995) Evidence for a unique chain organization in long chain silane monolayers deposited on two widely different solid substrates. Langmuir 11:2357–60
12. Geerken MJ, van Zanten TS, Lammertink RGH, Bornemann Z, Nijdam W, van Rijn CJM, Wessling M (2004) Chemical and thermal stability of alkylsilane based coatings for membrane emulsification. Adv Eng Mater 6:749–54
13. Grodrian A, Metze J, Henkel T, Martin K, Roth M, Köhler JM (2004) Segmented flow generation by chip reactors for highly parallelized cell cultivation. Biosens Bioelectron 19:1421–8
14. Martin K, Henkel T, Baier V, Grodrian A, Schön T, Roth M, Köhler JM, Metze J (2003) Generation of larger numbers of separated microbial populations by cultivation in segmented-flow microdevices. Lab Chip 3:202–7
15. Carlier J, Chuda K, Arscott S, Thomy V, Verbeke B, Coqueret X, Camart JC, Druon C, Tabourier P (2006) High pressure- resistant SU-8 microchannels for monolithic porous structure integration. J Micromech Microeng 16:2211–9
16. Sato H, Matsumura H, Keino S, Shoji S (2006) An all SU-8 microfluidic chip with built-in 3D fine microstructures. J Micromech Microeng 16:2318–22
17. Jenke MG, Schreiter C, Kim GM, Vogel H, Brugger J (2007) Micropositioning and microscopic observation of individual picoliter-sized containers within SU-8 microchannels. Microfluid Nanofluid 3:189–94
18. Voskerician G, Shive MS, Shawgo RS, von Recum H, Anderson JM, Cima MJ, Langer R (2003) Biocompatibility and biofouling of MEMS drug delivery devices. Biomaterials 24:1959–67
19. Christensen TB, Pedersen CM, Gröndahl KG, Jensen TG, Sekulovic A, Bang DD, Wolff A (2007) PCR biocompatibility of lab-on-a-chip and MEMS materials. J Micromech Microeng 17:1527–32
20. Schumacher JT, Grodrian A, Kremin C, Hoffmann M, Metze J (2008) Hydrophobic coating of microfluidic chips structured by SU-8 polymer for segmented flow operation. J Micromech Microeng 18:055019
21. Motamedi ME (2005) Micro-opto-electro-mechanical-systems. ISBN 0-8194-5021-9 1
22. Stubenrauch M, Fischer M, Kremin C, Hoffmann M, Müller J (2007) Bonding of silicon with filled and unfilled polymers based on black silicon. Micro Nano Lett 2(1):S6–8
23. Leopold S, Kremin C, Ulbrich A, Krischok S, Hoffmann M (2011) Formation of silicon grass: nanomasking by carbon clusters in cyclic deep reactive ion etching. J Vac Sci Technol B 29 (1):10.1116/1.3521490
24. Sinzinger S, Jahns J (2003) Microoptics. Wiley, Weinheim. ISBN 978-3-527-40355-4
25. Jahns J, Huang A (1989) Planar integration of free—space optical components. Appl Opt 28:1602–1605
26. Sinzinger S, Jahns J (1997) Integrated micro-optical imaging system with a high interconnection capacity fabricated in planar optics. Appl Opt 36:4729–4735
27. Barbieri R, Benabes P, Bierhoff Th, Caswell JJ, Gauthier A, Jahns J, Jarczyski M, Lukowicz P, Oksman J, Russell GA, Schrage J, Snowdon JF, Stübbe O, Troster G, Wirz M (2008) Design and construction of the high-speed optoelectronic memory system demonstrator. Appl Opt 47:3500–3512
28. Testorf M, Jahns J (1999) Imaging properties of planar-integrated micro-optics. J Opt Soc Am A 16:1175–1183
29. Stoebenau S, Sinzinger S (2009) Ultraprecision machining techniques for the fabrication of freeform surfaces in highly integrated optical microsystems. SPIE-conference "Optical Manufacturing and Testing VIII", San Diego. Proc SPIE 7426:742608
30. Jahns J, Sinzinger S (2000) Microoptics for biomedical applications. American Biotechnol Lab 11, Oktober 52–54

31. Amberg M, Stoebenau S, Sinzinger S (2010) Integrated free-space optical fluorescence detector for microfluidic applications. SPIE Proc. 7716–27
32. Amberg M, Stoebenau S, Sinzinger S (2010) Single-step replication of a highly integrated PDMS optofluidic analysis system. Appl Opt 49:4326–4330
33. Stoebenau S, Kleindienst R, Kampmann R, Sinzinger S (2011) Enhanced optical functionalities by integrated ultraprecision machining techniques (keynote paper). In: 11th international conference of the European Society of Precision Engineering and Nanotechnology (EUSPEN), Lake Como, Italy. 23.5.-27.5.2011
34. Kleindienst R, Stoebenau S, Kampmann R, Sinzinger S (2011) Hybrid optical (Freeform) components – functionalization of non-planar optical surfaces by direct ps-laser ablation. Appl Opt 50:3221–3228
35. Ashkin A, Dziedzic JM, Bjorkholm JE, Chu S (1986) Observation of a single-beam gradient force optical trap for dielectric particles. Opt Lett 11(5):288–290
36. Grier DG (2003) A revolution in optical manipulation. Nature 424(6950):810–816
37. Ashkin A (1970) Acceleration and trapping of particles by radiation pressure. Phys Rev Lett 24 (4):156–159
38. Rodrigo PJ, Kelemen L, Palima D, Alonzo CA, Ormos P, Glückstad J (2009) Optical microassembly platform for constructing reconfigurable microenvironments for biomedical studies. Opt Express 17(8):6578–6583
39. Thalhammer SBG, Steiger R, Ritsch-Marte M (2011) Optical macro-tweezers: trapping of highly motile micro-organisms. J Opt 13(4):044024
40. Sinzinger S, Amberg M, Oeder A, Hein D (2007) Integrated micro-opto-fluidic systems for optical manipulation of cell cultures. OSA Annual Meeting, Frontiers in Optics, San Jose, CA. USA, 16.9.-20.9.2007
41. Talbot HF (1836) Facts relating to optical science. Phil Mag 9
42. Fournier J-M, Rohner J, Jacquot P, Johann R, Mias S, Salathé R-P (2005) Assembling mesoscopic particles by various optical schemes. Proc of SPIE, 59300Y-1
43. Oeder A, Stoebenau S, Sinzinger S (2011) Free-form optical trapping systems. In Optical Trapping Applications, OSA Technical Digest (CD) (Optical Society of America, 2011), paper OTTuC2p (Postdeadline paper) http://www.opticsinfobase.org/abstract.cfm?URI=OTA-2011-OTTuC2p