Lab-on-a-Chip Systems with Integrated Optics for Biochemical Applications

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Abstract: *The challenges and possibilities of integrating optical elements with fludic channels for applications in miniaturized biochemical devices are discussed with the help of examples from our lab.*

Introduction

Over the past 15 years, a new field of research has emerged at the junction between chemistry, physics, biology and engineering. The objects of interest in this field are either referred to as micro-Total Analysis Systems (μ-TAS) or Lab-on-a-Chip Systems (LOC) [1,2]. Both build upon the ideas of gaining advantages by miniaturizing and integrating all functions necessary to perform a given (bio)chemical analysis and utilizing the particular features of fluids confined in micrometer-sized geometries (microfluidics). At these length scales, liquid flows are very well behaved, allowing to easily create laminae flowing alongside each other, which can then be used to control location, and perform flow sorting and other fluidic handling steps. Transport of chemical species across these laminae is mostly relying on diffusion, which, in most cases, is a fast enough process on small length scales, and thus another reason to miniaturize.

LOCs are being designed for applications in chemistry, biology and medicine, where often investigations have to be performed on very small amounts but at the same time on a large number of different samples. Here, the reduced consumption of chemicals and the improved performance in terms of time and efficiency are the main motivations to invoke miniaturization and microfluidics.

Traditionally, the design and fabrication of these devices has relied on techniques inherited from microelectronics and micromechanics. Thus, silicon and glass have been the preferred materials for realizing LOCs. However, in recent years, the focus has shifted towards polymer substrates, since these materials are potentially both better suited and cheaper for one of the main applications of LOCs, namely as one-time use diagnostic devices. For this to be successful, a range of new fabrication approaches and techniques had to be developed and tested. New designs making use of the special properties of polymers while considering their individual restrictions are continuously being developed.

All LOCs are made up of a number of functional elements, which are necessary for a given task. These typically include fluidic handling elements (channels, valves, pumps, …) and detection elements (electrodes, optical elements, sensors, …). Also here, the integration of several of these functional elements on a planar substrate in a miniaturized format can lead to performance improvements and, in fact, to new, hitherto unseen functionalities as compared to more conventional setups.

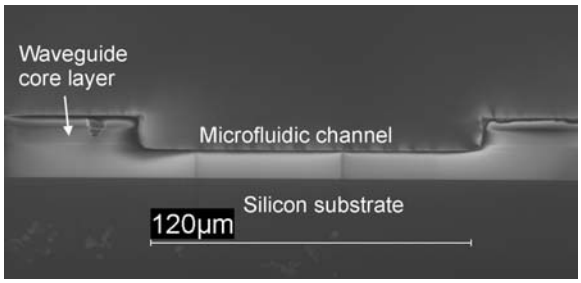

Fig. 1: Cross-sectional view of a fluidic channel with waveguides. The waveguides structure consists of three layers: a buffer layer of silicon oxide, a core layer of doped silicon oxide and a cladding layer of silicon oxide again. Note, no cover lid has yet been bonded to the structure.

In our lab, for a number of years now we have been working with integration of fluidic and optical elements, in an effort to steer away from bulk optics, which apart from taking up too much space, requires alignment before every new measurement and is not considered conducive to portable, hand-held devices. The central element in this effort was and is the waveguide, very well suited for the quasi 2D nature of planar microfluidic devices.

Nitrogen-doped and pure silica waveguides

To present on-chip alternatives to fluorescence detection and the often required labeling, sensitive absorbance detection on a chip is an option. Here, light is shone through the sample and the reduction of the measured light intensity is related to the concentration of analyte molecules present in the sample volume. While fluorescence can be very selective, absorbance (especially when including the UV portion of the optical spectrum) is almost universal, i.e., many different types of molecules can be directly detected without having to modify them prior to the detection. The main drawback is a drastically reduced sensitivity when compared to fluorescence detection, and – at first glance – implementing this technique in microsystems seems to be the wrong strategy. The measured absorbance, A, is, according to Beer's law, directly proportional to the optical path length, d:

$$A = \varepsilon c d$$

with c: concentration and ε: molar extinction coefficient. Without invoking more advanced machining, bulk optics approaches can only exploit the channel

depth as the optical path length, which in many cases is smaller than the channel width. Using

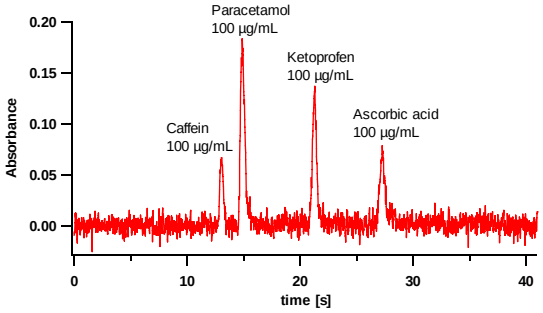

Fig. 2: Electrophoretic separation of four components in a microfluidic chip using UV-absorbance detection at 254 nm. The detection cell was the 750 um long lower part of a U-shaped channel probed by UV-transparent waveguides [6].

planar waveguides monolithically integrated with the fluidic channels, light can be brought in from the side thereby using the width of the channel as optical path length. Furthermore, U- and Z-cells can now be designed featuring even longer optical path lengths.

At MIC we have developed and fabricated UV-transparent waveguides using silica-on-silicon technology (see Figs. 1, 4). Changing the traditionally used germanium as core dopant material to nitrogen, waveguides with transmission down to about 210 nm were realized [3]. More recently, pure silica waveguides have been realized that show very low propagation losses down to 200 nm [4]. Using such waveguides microchips for capillary electrophoretic separation and UV-absorbance detection were realized – a typical separation is shown in Fig. 2 [5, 6] One of the practical challenges is still the optical connection to the macro-world, which is done via optical fibers. Since the cross-sections of fibers and waveguides differ, there is always the threat of stray light, i.e., light coming out of the fiber and propagating unguided through the buffer or cladding layer of the waveguide structure. Such light can reach the detector without interacting with any sample solution thereby limiting the performance of the detection set-up. Special care has to be taken in the design of the waveguides to avoid influences from stray light. This is also illustrated in the next example.

Polymer waveguides

With standard cleanroom technology silica-on-silicon waveguides can be fabricated with great accuracy and excellent optical qualities. However, some of the processes involved are rather time-consuming, making these waveguides more expensive and the turn-around time longer. Along with the generally increased interest in low-cost polymer fluidic devices there is a demand for waveguides and other optical elements made from polymeric materials as well. One polymer that is being used extensively in microsystems already is SU-8, which can be structured in a photolithographic process. Since it also has a sufficiently high refractive index, it is at the same time suitable as a waveguide. When using air as the cladding material at both sides it is possible to reduce the fabrication effort to just one mask step, and yet allowing the realization of fluidic channels, waveguides and fiber couplers (for easier connection

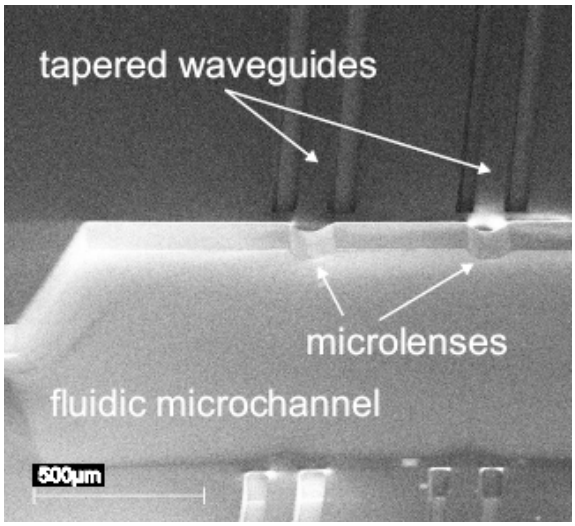

Fig. 3: Microfluidic channel with tapered waveguides and microlenses at the interface between channel and waveguides. All elements are defined in a single mask and realized using SU-8 lithography.

to the macro-world) at the same time [7]. It should, however, be noted that SU-8 has different optical properties than silica. It is only transparent down to around 580 nm, and is therefore preferably operated with red laser light. Using special detection geometries, optical path lengths of up to 1000 µm are employed, again in order to improve the detection sensitivity. However, this detection path length cannot be made arbitrarily long, as the signal-to-noise level deteriorates with a decreasing signal. It is therefore more important to ensure that as much as possible of the optical power delivered by the waveguide is used to interact with the molecules in the detection zone. In other words, the spreading of the (unguided) light within the channel has to be reduced. This can be achieved by shaping the beam as it exits the waveguide endface. SU-8 proves here to be a flexible material to work with, as it allows to taper the waveguides and even makes it possible to define lens structures at the interface between the waveguide and the fluidic channel (see Fig. 3) [8].

While working with SU-8 allows faster turnaround times and easier prototyping than working with silicon and glass, it is still a photolithographic process, which has only limited applicability for production of large numbers of replicas. Recently, micro-milling and casting processes have been employed to realize chips including waveguides in a potentially mass-producible way. Here, polymethylmethacrylate (PMMA) is used as the base polymer, whereas the higher refractive index necessary for waveguiding is ensured by a spun-on or cast PMMA layer, which is

doped with a different polymer to adjust its refractive index and subsequently structured to define the waveguides [9].

Fig. 4 presents an overview of the spectrally resolved propagation loss of all the different waveguides developed and tested in our lab over the past couple of years. Note, that the micro-milled waveguides have very acceptable propagation losses over a wide region of wavelengths.

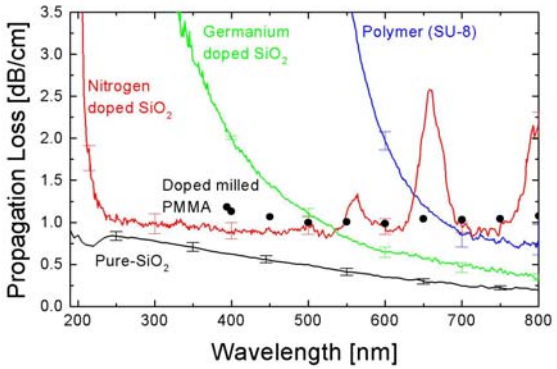

Fig. 4: Comparison of the wavelength-resolved propagation losses of a number of different waveguides fabricated and tested at MIC over the past several years.

Applications

As already mentioned, waveguides are combined with fluidic channels as detecting elements in separation systems (see Fig. 2). Here, one input and one output waveguide are typically sufficient. In principle, it is just as difficult to fabricate one waveguide as it is to fabricate an array of waveguides. With more than one waveguide, a number of applications can be realized, such as, e.g., velocity measurements on fluorescently labeled particles or cells. There exists a variety of detection principles, which can favorably be implemented on microchips with integrated waveguides, since this assures a high degree of accuracy in geometry and alignment. One such technique is known as Shah Convolution Fourier Transform detection (SCOFT). It was previously demonstrated on microchips using slitmasks and bulk optics [10,11], while the realization with waveguides is conceptually much more elegant and promises improved performance [12]. Figure 5 shows the layout of a chip developed for SCOFT including beamsplitters and an array of 32 waveguides (designs with up to 128 waveguides were tested). The goal of this layout was to achieve equally-spaced illumination of the fluidic channel.

Using such waveguide arrays velocities of particles and cells can be measured even when there is a continuous influx of particles in the detection area and also when ensembles of particles with different velocities are present at the same time. The velocity information is extracted from the recorded signal trace after a Fourier transform operation, which yields a frequency that is directly related to the velocity of the particles [12].

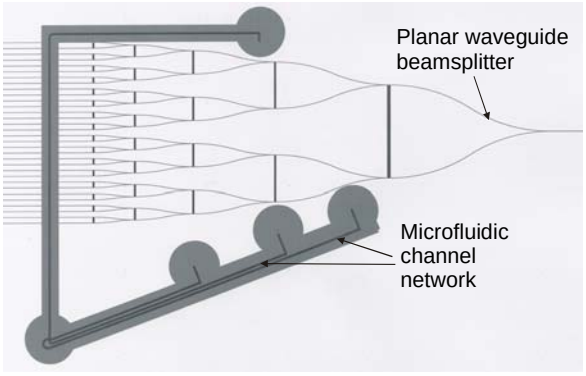

Fig. 5: A fluidic structure combined with an array of waveguides for periodic illumination of the channel, as used for the determination of the velocities of particles moving through the channel. The structures in between the split pairs of waveguides are stray-light rejection elements.

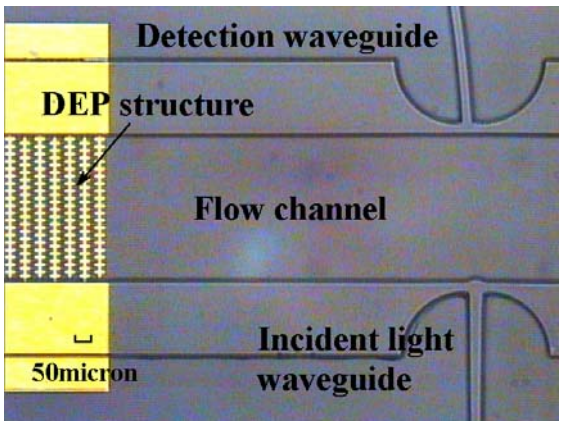

Fig. 6: A waveguide-based flow cytometer down-stream from a dielectrophoretic cell trap. Note the beam-shaping lens at the end of the incident light waveguide ensuring light to be focused in the path of the cells [14].

Waveguides can also be used in connection with fluidic channels to efficiently monitor passage of cells in these channels (flow cytometry) and even get further information on the cells or particles (such as size, viability etc.) using scattered light measurements [13]. Such measurements have been employed to gauge and better understand the function and efficiency of an on-chip dielectrophoretic (DEP) cell trap. By using a waveguide-based flow cytometer both upstream and downstream from the trap, its efficiency under different conditions can be monitored counting the cells passing each of the detection regions. Fig 6 shows the waveguide arrangement downstream from a DEP trap in a microfluidic channel [14].

Integration of light sources and detectors

Our efforts in fabricating waveguides to control where light is delivered and collected on a microfluidic chip were recently combined with other research

efforts going on at MIC. There has been work on the integration of photodiodes with particular emphasis on the connection and packaging issue when combining photodiodes and fluidic structures for aqueous chemistry. Also, there have been recent efforts to realize fluidic dye lasers on microchips at MIC. Here, a laser cavity was defined by SU-8 microstructures and rhodamine dye was percolated through the cavity while an Excimer laser acted as optical pump source. The combination of such a dye laser with waveguides, fluidic channels and photodiodes represents a highly integrated microsystem for biochemical applications. Figure 7 shows a picture of such a device and the layout beneath. The mixing device allows two chemicals to be mixed and the subsequent reaction can then be monitored by any or all of the waveguides spanning the fluidic channel at different positions along the flow direction [15].

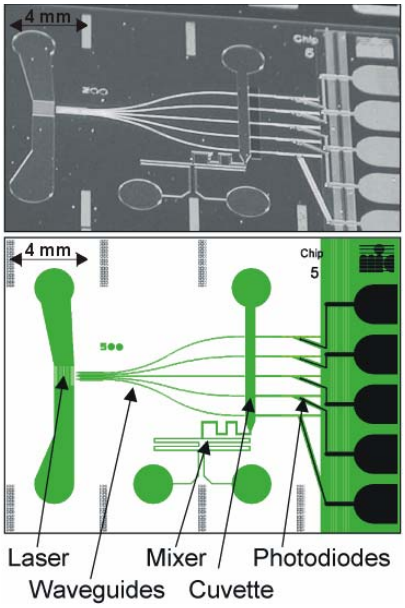

Fig. 7: Photograph (above) and layout (below) of a chip including fluidic channels, a fluidic dye laser, waveguides, and photodiodes as well as metallic pads for electrical connections [15].

Trends - Optofluidics

Optofluidics is an emerging field where nanophotonics and microfluidics meet. At MIC, we are developing microfluidic systems containing micro- or nanomachined elements that can fulfil both fluidic (chemical) functions as well as optical functions. Fig. 8 shows a chip containing a regular arrangement of pillars in a channel. This arrangement provides the function to separate complex mixtures of chemicals as they flow along the channel. At the same time, the arrangement constitutes an inverse photonic band gap structure and can thus be exploited for a number of different detection purposes. Light is coupled in perpendicularly to the flow direction using waveguides. Pick-up waveguides are also placed on the opposite side of the channel. With such an arrangement, detection can be performed at various stages along the flow direction, thus also allowing the dynamics of a separation or a reaction to be monitored. It is expected that this and similar structures will introduce new detection schemes for LOCs.

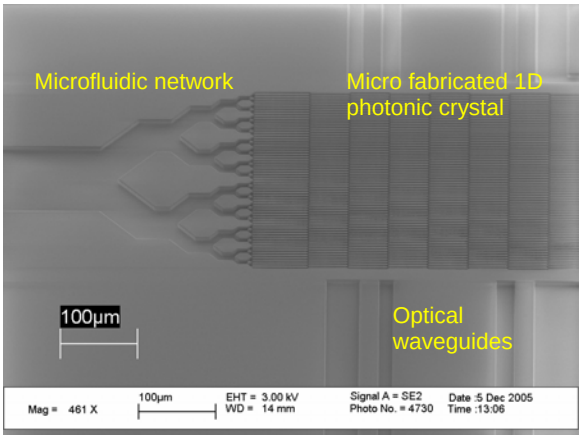

Fig. 8: Micrograph of a fluidic structure with a regular array of pillars, which at the same time can fulfil fluidic and optical functions.

Conclusions

Integration of optics and fluidics offers a range of possibilities for new and improved tools for applications in the life sciences.

Acknowledgments

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