Fluorescence excitation on monolithically integrated all-polymer chips

Mauno Schelb*
Christoph Vannahme
Karlsruhe Institute of Technology
Institut für Mikrostrukturtechnik
Kaiserstrasse 12
Karlsruhe, 76131 Germany

Alexander Welle
Karlsruhe Institute of Technology
Institut für biologische Grenzflächen
P.O. Box 3640
Karlsruhe, 76128 Germany

Steven Lenhert
Karlsruhe Institute of Technology
Institut für Nanotechnologie
Karlsruhe, 76128 Germany
  and
Florida State University
Department of Biological Science and
Integrative NanoScience Institute
Tallahassee, Florida 32306-4370

Benjamin Ross
Timo Mappes*
Karlsruhe Institute of Technology
Institut für Mikrostrukturtechnik
Kaiserstrasse 12
Karlsruhe, 76131 Germany

Abstract. All-polymer chips with integrated optical waveguides and microfluidic channels were built as a platform for fluorescence excitation of biological samples. Their functionality has been shown for four cases: (i) fluorescence of labeled phospholipids inside a microfluidic channel, (ii) fluorescence of stained cells inside a microfluidic channel, (iii) fluorescence of labeled phospholipids on top of a polymer waveguide, and (iv) fluorescence of stained cells on top of a polymer waveguide. © 2010 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3446672]

1 Introduction

Fluorescence excitation and observation is a common technique of investigation in the field of biochemistry and cell biology. In our approach, we demonstrate the excitation of fluorescence on an integrated optofluidic system.[1] Microfluidic systems can be used as analysing platforms requiring only a minimum amount of biological or chemical analyte. By the use of microoptical waveguides, specific spots of analyte or cells can be addressed optically,[2–4] which makes the combination of waveguides and fluorescence microscopy a useful tool for cell biology.[5–7] The implementation of fluorescence detection in such a microfluidic and microoptical system may open up the possibility for, e.g., cell based sensors, where it is often advantageous to investigate a very limited amount of a certain cell culture during the study of its behavior and characteristics.

The integration of optical waveguides and channels in one system has been shown for a combination of different materials, for instance, glasses and poly(dimethylsiloxane) (PDMS),[8] glasses and silicon,[9] and polymers and silicon.[10] In our system, we combined microfluidic channels and microoptical waveguides on a single polymer substrate made from poly(methyl methacrylate) (PMMA). This approach is favorable due to biocompatibility, optical properties, fabrication flexibility, and the low cost of the raw material.[11] After fabrication of the chip, stained living animal cells or fluorophore-labeled phospholipids were deposited onto the planar chip or into a microfluidic channel in the chip. The fluorescent labels were excited by light coupled into the integrated waveguide and guided to the cells or phospholipids. Emitted fluorescence was observed with a microscope.

2 Chip Layout

For the implementation of a fluorescence measurement on an integrated polymer chip, two different types of fluorescent medium and two different layouts were set up (Fig. 1). The two media are fluorescently labeled phospholipids [Figs. 1(a) and 1(c)] on the one hand and fluorescently stained cells [Figs. 1(b) and 1(d)] on the other hand. Those were either introduced into a channel crossing an optical waveguide [Figs. 1(a) and 1(b)] or directly applied on top of an optical waveguide [Figs. 1(c) and 1(d)].

Address all correspondence to: Mauno Schelb, Institut für Mikrostrukturtechnik, Karlsruhe Institute of Technology, Kaiserstrasse 12, Karlsruhe, 76131 Germany. Tel: 49-7247-82-6839; Fax: 49-7247-82-4331; E-mail: mauno.schelb@kit.edu
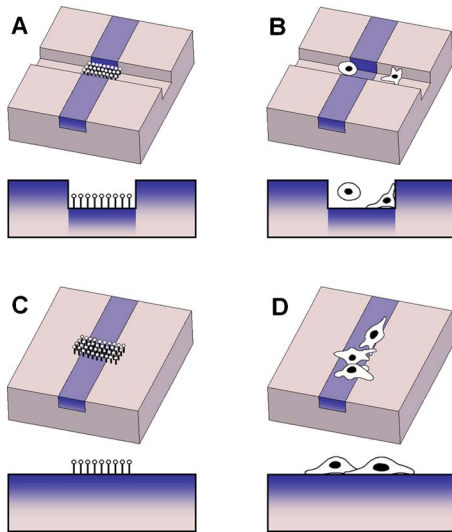*These authors contributed equally to this paper.

Fig. 1 Schemes of the different setups used for fluorescence excitation on an all-polymer chip (optical waveguide in blue): (a) Phospholipids in a microfluidic channel excited directly via waveguide, (b) cells in a microfluidic channel excited directly via waveguide, (c) phospholipids on top of a waveguide excited by evanescent field, and (d) cells on top of a waveguide excited by evanescent field. (Color online only).

3 Fabrication

The two layouts were realized on a polymer substrate. Here, photochemical degradation of PMMA by deep ultraviolet light (DUV) is used to process the channels and optical waveguides (Fig. 2).

The microchannels in setups A and B were generated by DUV lithography and subsequent development. In a first step, the channel structures are patterned by conventional shadow-printing lithography using a quartz-chromium mask. Bulk PMMA (Hesa®Glas by NotzPlastics) was used as semifinished product and exposed to DUV radiation [Fig. 2(b)] with a commercial UV-lithography system (maskaligner EVG 620) operated at a wavelength of 240–250 nm. The DUV-exposed
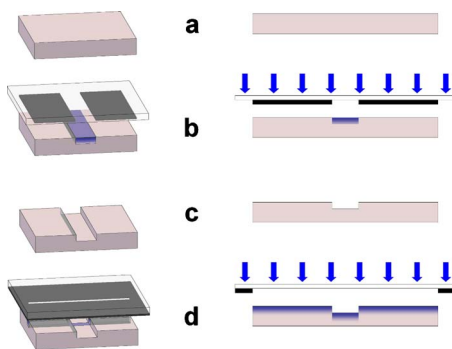

Fig. 2 Fabrication steps for integrated polymer waveguides and microfluidic channels in a monolithic system: (a) Semifinished product: PMMA sheet of 1 mm thickness, (b) photodegradation of the PMMA to form the trench: deep ultraviolet lithography ($\lambda = 240$–250 nm) through a quartz-chromium mask, exposure dose 4–8 J/cm^2, (c) subsequent development of the 4–8 μm deep trench with MIBK/IPA,[9] and (d) creation of the polymer waveguide perpendicular to the trench: deep ultraviolet lithography through a quartz-chromium mask, exposure dose $\sim$3 J/cm^2.

PMMA is photochemically degraded (i.e., molecular chains of the polymer are cracked such that the average molecular weight is reduced). This reduction leads to alteration of chemical and optical properties of the exposed PMMA. Because of the altered solubility of the irradiated PMMA, the exposed areas can be dissolved using a developer. In our case, an exposure dose of 4–8 J/cm^2 was applied and the development of the irradiated PMMA was carried out using methyl isobutyl ketone (MIBK) and isopropanole (IPA),[12] resulting in a trench of 4–8 μm depth [Fig. 2(c)].

An exposure dose of $\sim$3 J/cm^2 applied with the same UV-lithography system was chosen to create the optical waveguides. Because of the DUV exposure, the irradiated PMMA shows a refractive index increase of $\sim$0.005 refractive index units in comparison to the refractive index of 1.48 for the substrate. The irradiated PMMA can be used as core of an optical waveguide[13] with a graded index profile and an exponential decay in vertical direction.

In our case, the optical waveguides were structured into the PMMA substrate as lines of 5 or 7.5 μm width by DUV lithography. For setups C and D, the waveguides were patterned into the bulk PMMA substrate while for setups A and B, they were patterned perpendicularly to the microfluidic channels.

After the patterning of microchannels and optical waveguides, the chip was mounted on a transparent polymer ground plate. This groundplate was used as support for fixing a glass fiber with glue in a way such that light could be coupled into the waveguide. Fixed on the ground plate and coupled to the glass fiber, the optofluidic chip could be used for the culturing of cells and the following experiments under a conventional fluorescence microscope.

4 Labeling and Observation of Fluorescence

After fabrication of microfludic channels (setups A and B) and optical waveguides in PMMA the chips were tested with fluorescently labeled phospholipids (cases A and C) or cells (cases B and D).

The labeled phospholipids were deposited via Dip-Pen nanolithography (DPN), which can be regarded as a modified and parallelized atomic force microscopy (AFM) setup.[14,15] Here, the AFM tips work similar to a pen, taking up a solution of phospholipids. By directing the AFM-tips onto the polymer surface, the phospholipid can be deposited with a lateral precision of several hundred nanometers. Thus, this method is particularly suited for deposition of phospholipid into, e.g., microfluidic channels as in our case.[16]

For the fluorescence measurement, the PMMA chip was mounted on a polymer base plate. A glass fiber was fixed on the base plate in order to allow stable end fire coupling to the optical waveguide on the chip. For fluorescence excitation light was coupled into the waveguide and fluorescence detected with a microscope objective (Fig. 3).

For setup A, biotinylated phospholipids (18:1 Lissamine Rhodamine PE, Avanti No. 810150, Avanti Polar Lipids Inc.) were deposited inside the microfluidic channel in the intersection of channel and waveguide, forming a spot of lipids on the bottom of the channel [Fig. 1(a)]. Subsequently, streptavidin (Cy-3, Product No. S6402-1ML, Sigma-Aldrich Co.) labeled with the fluorescent dye Cy3 (absorption maximum 550 nm,
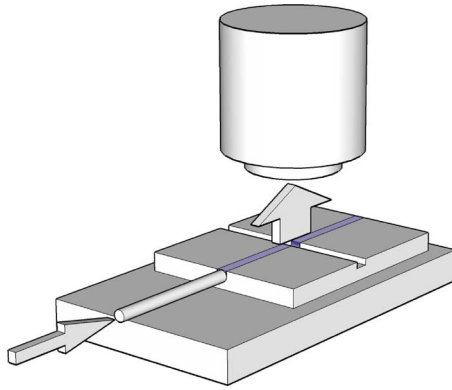
Fig. 3 Scheme of the experimental setup. The polymer chip is mounted on a base plate, and a glass fiber is coupled to the optical waveguide. The excitation light is coupled from the the glass fiber (left arrow) to the integrated waveguide. Fluorescent light is detected through the objective of an optical microscope (vertical arrow).

emission maximum 570 nm) was bound to the biotinylated lipids. After depositing Cy3-labeled lipids into the channel-waveguide intersection by DPN, fluorescence was excited by green light and observed with a fluorescence microscope. In a first step, a reference image was taken with a Nikon TE 2000 microscope using a mercury source filtered for excitation at 540–580 nm. Emission was detected at 600–660 nm [Fig. 4(a), second column]. Then, the fluorescent labels were excited by green laser light (wavelength 532 nm), which was coupled to and guided through the polymer waveguide directly to the channel-waveguide intersection. Herewith, the part of the phospholipid spot directly located in the intersection area could be excited selectively by the light coupled out of the end-facet of the polymer waveguide inside the channel [Fig. 4(a), third column].

For setup B, the same experiment was carried out using living L929 mouse fibroblasts. The cells were cultured following a conventional culturing.[17] After trypsination, the cells were stained in suspension with DiD Vybrant™ Cell-Labeling
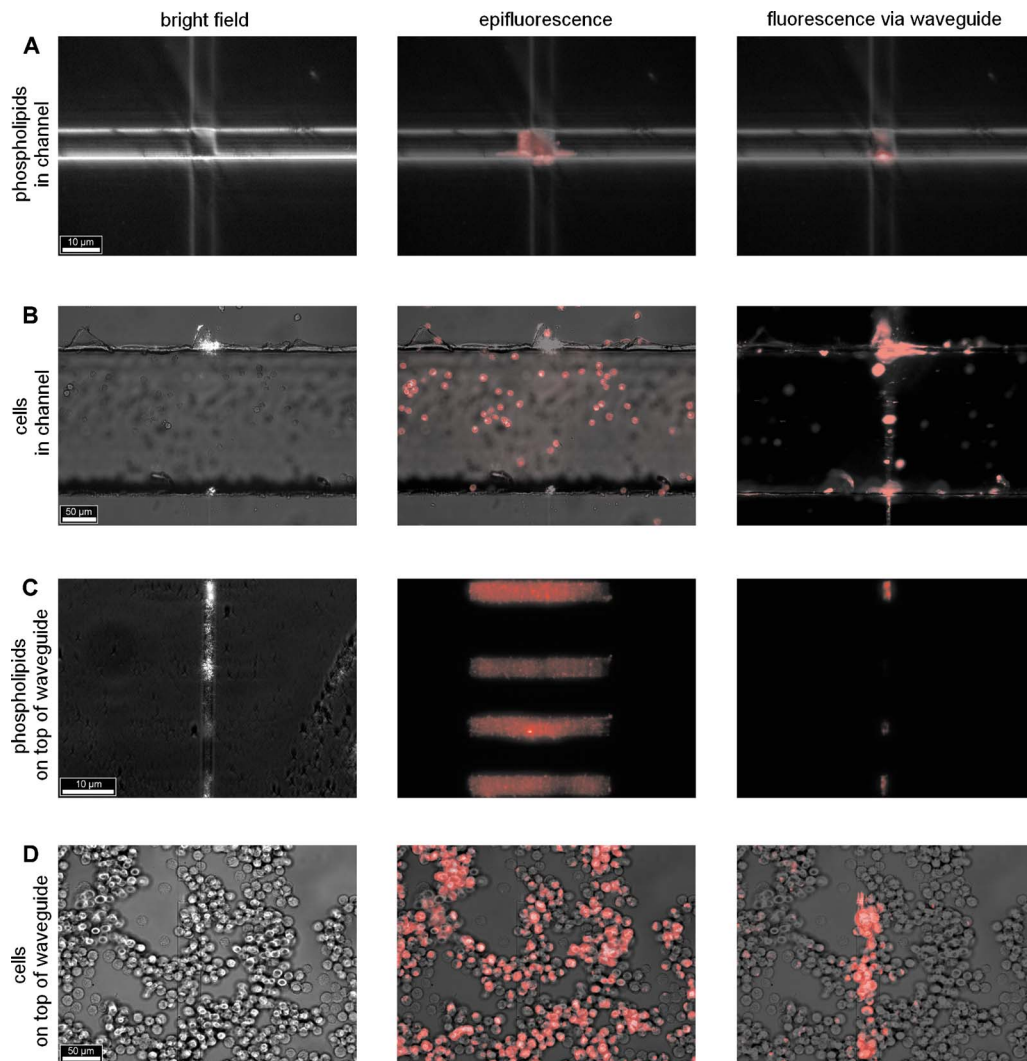

Fig. 4 Micrographs for the setups (a), (b), (c), and (d). The first image in each row shows a bright field image of each specific setup. The polymer waveguide is oriented vertically, the fluidic channel crosses perpendicularly (a and b). The second image shows a fluorescence image of the same area under flood light excitation of fluorescence via the microscopy optics. The third image in a row shows a fluorescence image where the fluorescent dye is excited via the polymer waveguide only (all images in pseudocolor). (Color online only).
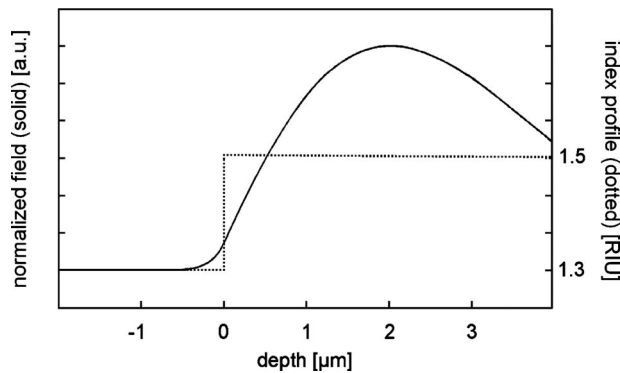
Fig. 5 Waveguide mode (solid line) simulated by the beam propagation method and corresponding refractive index profile (dotted line) of a polymer waveguide.

solution (Catalogue No. V-22887, Molecular Probes), according to the manufacturers recommendations. This lipophilic carbocyanine dye (absorption maximum 650 nm, emission maximum 670 nm) selectively stains the cell membrane. After washing the cell suspension to remove unbound dye, the cell suspension was introduced into the channel and microscopy images were recorded while the cells remained in suspension. Results from this experiment are similar to the one with labeled phospholipids. Under flood exposure in the wavelength range of 630–640 nm with a Zeiss Apotome, all cells floating through the channel were excited. The emitted fluorescent light was detected at 650–690 nm [Fig. 4(b), second column]. Coupling red laser light (wavelength 635 nm) into the waveguide allowed to excite solely the cells crossing the laser light beam at the moment of capture [Fig. 4(b), third column].

For setup C, DPN was carried out depositing phospholipids labeled with Rhodamine B (absorption maximum 560 nm, emission maximum 570 nm) directly onto a polymer waveguide [Fig. 1(c)]. In this case (as well as in setup D), our goal was to excite the fluorescent labels via the evanescent field of the optical mode propagating through the waveguide buried in the polymer. The evanescent field of the waveguide mode extends a few hundred nanometers into the medium above the polymer (Fig. 5). Fluorescence was excited by flood exposure with the same microscope setup as in case A as a reference measurement. The labeled spot of phospholipids is clearly visible [Fig. 4(c), second column]. Afterward, green laser light (wavelength 532 nm) was coupled into the waveguide, in order to excite the phospholipids via the evanescent field. Solely, the lipids directly covering the waveguide were visible in the fluorescence microscope [Fig. 4(c), third column]. Hence, the evanescent field portion is sufficient to excite the fluorescent labels in the lipid layer.

In setup D, we wanted to visualize fluorophore stained cells via the evanescent field of a waveguide mode. Thus, the mouse fibroblasts stained with the DiD were allowed to adhere to the isopropanole washed polymer surface for three days. Although the cell number approximately doubled during this time due to cell proliferation, the staining was found to be still sufficient for imaging. However, the morphology of the fibroblasts was rather nonphysiological, indicating weak adhesion to the substrate, which might have to be improved by

surface modifications. After a measurement under flood exposure with the same microscope setup as in case B [Fig. 4(d), second column], the cells were excited by red laser light (wavelength 635 nm) guided along the underlying waveguide. As in case C, fluorescence could be excited selectively for the cells adhered directly above the waveguide [Fig. 4(d), third column].

5 Conclusions

We realized the visualization of fluorescence excited via waveguides in an integrated optofluidic microsystem for fluorescently labeled phospholipids and stained cells. The measurement is a proof of concept for the layout of a cell-based optofluidic microsensor built as a monolithic polymer device. The advantage of the presented layout compared to other waveguide excitation systems[5,6] is the lateral definition of the waveguide, which makes it possible to address groups of cells selectively. Applications of such a system could be the selective investigation of morphology changes and different cellular responses[2,18] as well as the detection of cells in separation systems or kinetics of binding events via monitoring fluorescence reactions.

The presented results could also be interesting for lab-on-a-chip systems. For the presented optofluidic system to be used as handheld device the microfluidic channels need to be covered. This can be accomplished by thermal bonding or laser welding of another polymer sheet on top of the optofluidic chip. Further steps on the road toward a handheld lab-on-a-chip system are the integration of light sources and fluorescence detectors. Regarding the integration of light sources meeting the requirements for low bandwidth fluorescence excitation, this could be realized by organic semiconductor lasers. Those lasers can be directly integrated into a PMMA substrate and optically pumped from the outside. The detection could be realized by application of photodiodes, which are combined with a filter just as the fluorescence filters used in the fluorescence microscopes. The filters could be realized integrating Bragg gratings that are patterned into a polymer waveguide, which is guiding the emitted fluorescent light from the analyte to the photodiode. The integration of those elements is subject of ongoing research in our group.

References

1. P. S. Dittrich, K. Tachikawa, and A. Manz, "Micro total analysis systems. Latest advancements and trends," *Anal. Chem.* **78**(12), 3887–3907 (2006).
2. J. Voros, R. Graf, G. L. Kenausis, A. Briunink, J. Mayer, M. Textor, E. Wintermantel, and N. D. Spencer, "Feasibility study of an online

toxicological sensor based on the optical waveguide technique," *Biosens. Bioelectron.* **15**, 423–429 (2000).

3. P. Lucas, A. A. Wilhelm, M. R. Riley, D. L. DeRosab, and J. M. Collier, "Cell-based bio-optical sensors using chalcogenide fibers," *Proc. SPIE* **6083**, 60830W (2006).

4. R. A. Seger, D. G. Rabus, Y. Ichihashi, M. Bruendel, J. Hieb, and M. Isaacson, "Monitoring fluorescence in cultured neural networks using polymer waveguide excitation," *Dig. IEEE/LEOS Summer Topics* 113–114 (2007).

5. B. Agnarsson, J. Halldorsson, N. Arnfinnsdottir, S. Ingthorsson, T. Gudjonsson, and K. Leosson, "Fabrication of planar polymer waveguides for evanescent-wave sensing in aqueous environments," *Microelectron. Eng.* **87**, 56–61 (2010).

6. H. M. Grandin, B. Städler, M. Textor, and J. Vörös, "Waveguide excitation fluorescence microscopy: a new tool for sensing and imaging the biointerface," *Biosens. Bioelectron.* **21**, 1476–1482 (2006).

7. L. N. Jiang, K. P. Gerhardt, B. Myer, Y. Zohar, and S. Pau, "Evanescent-wave spectroscopy using an SU-8 waveguide for rapid quantitative detection of biomolecules," *J. Microelectromech. Syst.* **17**(6), 1495–1500 (2008).

8. J. M. Ruano, A. Glidle, A. Cleary, A. Walmsley, J. S. Aitchison, and J. M. Cooper, "Design an fabrication of a silica on silicon integrated optical biochip as a fluorescence microarray platform," *Biosens. Bioelectron.* **18**(2–3), 175–184 (2003).

9. G. Arun, V. K. Sharma, A. Kapoor, and K. N. Tripathi, "Partially and fully cured thin film photoresist waveguides for integrated optics," *Opt. Laser Technol.* **34**(5), 395–398 (2002).

10. L. Malic and A. G. Kirk, "Integrated miniaturized optical detection platform for fluorescence and absorption spectroscopy," *Sens. Actuators, A* **135**(2), 515–524 (2007).

11. H. Ma, A. K. Jen, and L. R. Dalton, "Polymer-based optical waveguides: materials, processing, and devices," *Adv. Mater. (Weinheim, Ger.)* **14**(19), 1339–1365 (2002).

12. T. Mappes, J. Mohr, K. Mandisloh, M. Schelb, and B. Ross, "A compound microfluidic device with integrated optical waveguides," *Proc. SPIE* **6992**, 69920L (2008).

13. P. Henzi, D. G. Rabus, U. Wallrabe, and J. Mohr, "Fabrication of photonic integrated circuits by DUV-induced modification of polymers," *Proc. SPIE* **5451**, 24–31 (2004).

14. K. Salaita, Y. H. Wang, and C. A. Mirkin, "Applications of dip-pen nanolithography," *Nat. Nanotechnol.* **2**, 145–155 (2007).

15. S. Lenhert, P. Sun, Y. H. Wang, H. Fuchs, and C. A. Mirkin, "Massively parallel dip-pen nanolithography of heterogeneous supported phospholipid multilayer patterns," *Small* **3**, 71–75 (2007).

16. T. Mappes, S. Lenhert, O. Kassel, C. Vannahme, M. Schelb, and J. Mohr, "An all polymer optofluidic chip with integrated waveguides for biophotonics," *Dig. IEEE/LEOS Summer Topics* 215–216 (2008).

17. T. Scharnweber, C. Santos, R. P. Franke, M. M. Almeida, and M. E. Costa, "Influence of spray-dried hydroxyapatite-5-fluorouracil granules on cell lines derived from tissues of mesenchymal origin," *Molecules* **13**(11), 2729–2739 (2008).

18. M. N. Velasco-Garcia, "Optical biosensors for probing at the cellular level: a review of recent progress and future prospects," *Semin Cell Dev. Biol.* **20**(1), 27–33 (2009).