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Design and integration of a multi-channel fluorescence detector

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For examination in the bio-chemical processes miniaturized fluorescence analysis systems are required. Based on a previous integrated fluorescence detector concept, the second generation of the system is designed for improved multifunctionality and measuring accuracy. We present the concept, optical design and expected performance of this new integrated detection system.

1 Introduction

In combination with (micro-)fluidic systems, fluorescence detectors have a large variety of applications, e.g. in biological and medical research (Fig. 1, left). Fluorescence as indicator in bio-chemical processes is e.g. sensitive for measuring pH-value, temperature and substance concentration. Highly integrated and miniaturized optofluidic microsystems enable the dynamic measurement of very small sample volumes in so-called segmented flow systems, which is beneficial for practical applications.

If a fluorophore is illuminated with light of a specific spectral bandwidth, it excites fluorescence light in the sample, which usually has a longer wavelength than the illumination due to the so-called Stokes shift (Fig. 2, right). It allows emission light to be detected separately from excitation light. However, the difference between the peak of the absorption and emitting spectra of the fluorophore is just a few nanometers and both spectra normally overlap significantly. Therefore a shortpass filter in the excitation path which ensures excitation at the correct wavelength, and a longpass filter in the fluorescence path which avoids crosstalk of the excitation light onto the fluorescence detector are necessary for optimum separation of both signals. A detector, sensitive within the wavelength band of the fluorescence signal, is used for detection.

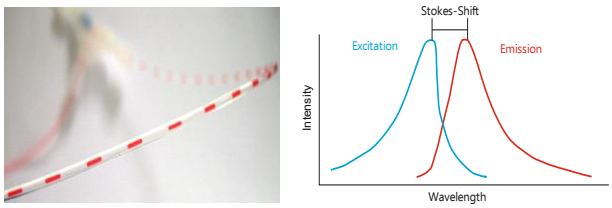

Fig. 1 *Left: Dye solution with carrier media Tetradecane as compartments in a tube. Right: Functional principle of fluorescence detection [1].*

2 Previous integrated system

Fig. 2 shows a single-channel fluorescence sensor using a planar integrated free space optical system, which has been developed in the past [2]. Monolithic fabrication was realized in a PMMA substrate by ultraprecision micromilling with a Kugler Microgantry™ nano5X ultraprecision machining center The integrated system including LED and detector was characterized experimentally in the lab. A fluorescence signal from Resorufin with a concentration of 250 µMol/l in 5 µl assay volume in PVC capillary could be detected [1].

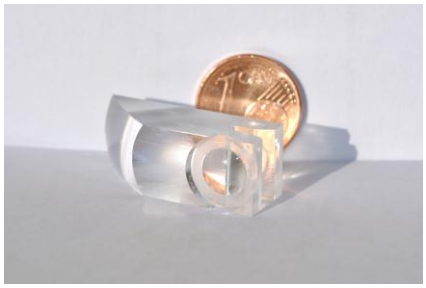

Fig. 2 *The fabricated fluorescence detector [2].*

In order to increase the measuring flexibility and accuracy as well as to satisfy more requirements, the extension to a multi-wavelength and multi-channel sensor system is currently investigated. Goals of the new system are 1) the detection of different substances, which are sensitive at different wavelengths, 2) minimizing signal fluctuation with a reference arm, 3) measuring turbidity and fluorescence simultaneously and 4) improved sensitivity and efficiency.

3 Design principle

The major challenge for the design, besides achieving all the functionalities, is to increase the systems efficiency, which means to maximize the signal directed onto the detector. Fig. 3 shows the principle set-up of the improved fluorescence detector. The LED source is collimated and split into two identical arms (one for the measurement and the other one as the reference) by the integrated biprism. Each excitation arm is focused by the parabolic mirror onto the fluid segments in the tube to illuminate the fluorescence markers. The center

of the tube is placed in the focus of the parabolic surface in order to realize a perfect focusing of the collimated light. This focal point which corresponds to the source point of the fluorescence light is at the same time one focus point of the elliptical surface. Thus the excited light from the fluoresceing probe can be focused by the elliptical mirror onto the detector located in the second focus point of the ellipse most efficiently. The signal is deflected and filtered before it reaches the detector. All the optical components are integrated and will also be fabricated as one monolithic PMMA-system.

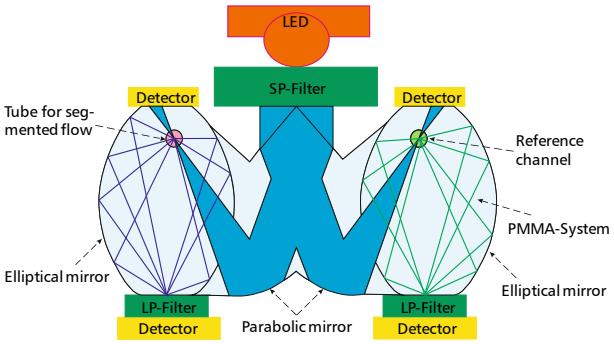

Fig. 3 *Schematic setup of the fluorescence detector.*

4 Modeling and simulation

For the optical design, the 3D-CAD software Solidworks™ and the raytracing tool ZEMAX™ were used. At first the whole detector including the PMMA-system, the tube and the fluid was modeled with Solidworks™ as shown in Fig. 4. After that all the components were imported separately into a ZEMAX file, with which the simulations were done. The excitation arm and the fluorescence arm were considered and simulated separately in ZEMAX, since there is no direct connection and influence between them.

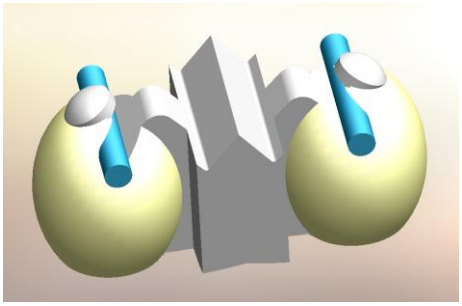

Fig. 4 *The detector model in Solidworks.*

In the first simulations an ideally collimated light source with a radius of 1.5 mm was used. Fig. 5 shows the simulation results in ZEMAX. The left spot diagram shows the focus of the excitation light on the segment. The spot size is about 0.03 mm x 0.001 mm, which is much smaller than the individual fluidic segments, and is asymmetric due to the PVC-tube, which works like a cylindrical lens. In the simulations of the fluorescence path a cylinder with r = 0.1 mm and l = 1 mm was used as the light source. These dimensions correspond to a typical extension of a fluidic segment. Fig. 5 right shows the fluorescence light distribution on the lower focus of the ellipse, i.e. the detector location. About 60% of the entire fluorescence light (1 W as the source) can be collected by the elliptical mirror. Due to the significantly extended useful area/angle to collect the fluorescence light in comparison with earlier systems, the efficiency and signal-to-noise ratio can be significantly increased.

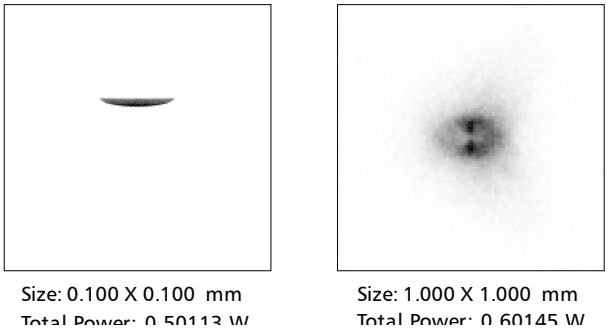

Fig. 5 *Simulation results in ZEMAX, left: focus of the collimated excitation light on the segment, right: the fluorescence light on the focus of the parabolic mirror.*

5 Conclusion and outlook

We presented the design concept of a planar integrated free space optical fluorescence detector in a segmented-flow environment. The design of the microoptical system was performed with the raytracing software tool ZEMAX™. Compared to the first generation system, the new system has more functionality and improved efficiency. Significant challenges however remain for the fabrication. The unique flexibility of ultraprecision milling process is currently exploited for this purpose.

Acknowledgements

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[2] M. Amberg, S. Stoebenau, S. Sinzinger, “Integrated free-space optical fluorescence detector for microfluidic applications”, SPIE Proc. 7716-27 (2010).