Enabling High-Resolution Fluorescence Microscopy and Detection using Integrated Photonics

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ABSTRACT

For many applications in life sciences, the biologically relevant information is probed by means of visible light. Many of the critical optical components have, unfortunately, still a large footprint and heavy price tag. Silicon nitride integrated waveguide optics –allowing for complex routing schemes of visible light across a chip– assumes a prominent role in the progressing miniaturization of optical devices. Here, we demonstrate how our recently developed integrated opto-fluidic chips, fabricated in a 200mm CMOS pilot line, allow aggressive miniaturization of cell imaging–*structured illumination fluorescence microscopy*, and in-flow cell counting–*cytometry*.

Keywords: microscopy, cytometry, structured illumination, fluorescence, silicon nitride, thermo-optic modulator

1. INTRODUCTION

1.1 Integrated photonics for structured illumination fluorescence microscopy

Fluorescence microscopy is an indispensable tool in biology and medicine, fuelling many breakthroughs in a wide set of sub-domains. Yet, the resolution is intrinsically restricted by the diffraction limit. The last two decades have witnessed the emergence of several super-resolution fluorescence microscopy techniques that are breaking this limit (cfr. Nobel Prize in Chemistry 2014). Structured illumination microscopy (SIM) is one of such techniques that has gained much popularity and is available in commercial systems. In SIM, a biological sample is illuminated by a spatially structured light field — a sinusoidal interference pattern — which causes normally inaccessible high-resolution information to be encoded into the observed image due to the Moiré effect. However, making use of free space optics, state-of-the-art SIM systems require multiple bulky and precision optical components that give these systems the drawbacks of high cost and cumbersome size. Here, we present a structured illumination microscopy system based on a photonic integrated circuit (PIC). For fluorescence imaging applications, a low loss and low auto-luminescence visible waveguide material is indispensable. Therefore, a plasma enhanced (PE) CVD silicon nitride process available at the imec 200mm SiN photonics pilot-line is used [1]. For active on-chip optical modulation, a thermo-optic phase shifter module was developed, providing a platform with full control over the phase and orientation of the sinusoidal illumination patterns at the sample (Figure 1). The sample is placed in direct contact with the PIC where the fluorophores are excited by the evanescent field of a slab waveguide mode. Analogues to conventional total internal reflection microscopy, this provides a very high surface sensitivity with high signal-to-background imaging. The unique properties of photonic integrated circuits allow us to create truly innovative microscopy systems that have the potential to go well beyond the current state-of-the-art: higher resolution due to the use of high refractive index materials, easier alignment with on-chip illumination light path, large field of view, a compact form factor resulting from on-chip integration, and lower cost due to compatibility with CMOS chip fabrication.

1.2 Integrated photonics for flow cytometry

Another life science application that can highly benefit from PICs is flow cytometry (cell counting). Here, target cells are fluorescently labelled. We present a miniature flow cytometry device based on on-chip integrated photonics and microfluidics. At its heart is a specially designed diffraction grating which delivers uniform fluorescence excitation with an extremely small footprint. Using integrated microfluidics, monolithically integrated in the same pilot line, the opto-fluidic system was characterized with calibration fluorescent polystyrene beads to characterize the cytometry functionality (Figure 2). This device concept allows dense parallelization and can constitute can become an elementary component for chip-based cytomics [2,3].

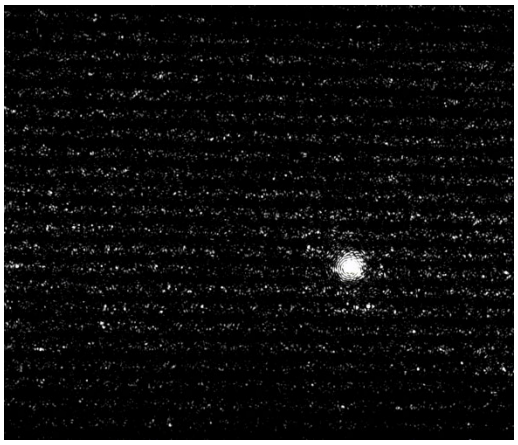

Figure 1. Bright field microscope image of the PIC slab waveguide showing one of the sinusoidal interference patterns used to generate a super-resolved image. The bright spot is a fluorescent bead.

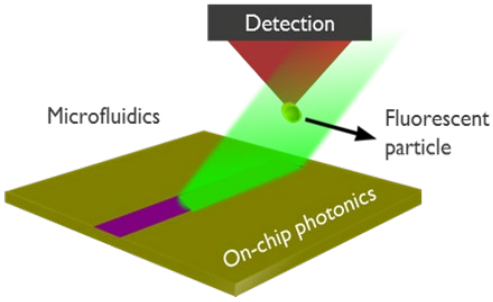

Figure 2. Illustration of the miniature cytometry device indicating the photonics chip with specially designed grating (purple), created uniform illumination (green), fluorescent particle flowing through the micro-fluidic channel after being hydrodynamically focused, and its luminescence being detected (red).

2. CONCLUSIONS

The unique properties of photonic integrated circuits allow us to create truly innovative optical systems. We investigate microscopy systems that have the potential to go well beyond the current state-of-the-art: higher resolution due to the use of high refractive index materials, easier alignment with on-chip illumination light path, large field of view, a compact form factor resulting from on-chip integration, and lower cost due to compatibility with CMOS chip fabrication. The latter advantages similarly apply to cytometry based on in-flow fluorescence detection. Here, the opportunity for micro-fluidic integration and dense parallelization of detection sites translates into unprecedented throughputs, with applications e.g. in cell manufacturing for cell therapies.

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