Frontiers in Optics + Laser Science (FiO/LS) @ Optica Publishing Group 2022

UV-compatible photonic integrated circuits for label-free structured illumination microscopy

Chupao Lin,[1,2,*] Juan Santo Domingo Peñaranda,[3] Jolien Dendooven,[3] Christophe Detavernier,[3] David Schaubroeck, [4] Nico Boon,[5] Roel Baets,[1,2] and Nicolas Le Thomas[1,2]

[1] *Photonics Research Group, INTEC Department, Ghent University-imec, Technologiepark-Zwijnaarde, 9052 Ghent, Belgium*

[2] *Center for Nano- and Biophotonics, Ghent University, Belgium*

[3] *Department of Solid State Sciences, CoCooN, Ghent University, Krijgslaan 281/S1, 9000 Ghent, Belgium*

[4] *Centre of Microsystems Technology (CMST), imec and Ghent University,Technologiepark 126, B-9052 Zwijnaarde, Belgium*

[5] *Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, Building A-9000, Gent, Belgium*

[*] *Chupao.Lin@UGent.be*

Abstract: There is a growing interest in photonic integrated circuits for biophotonic applications. Here, we present such a circuit operating in the ultraviolet that allows us to implement super-resolved label-free structured illumination on yeast cells.

1. Introduction

Integrated photonics is a key enabling technology that has already benefited various applications ranging from telecommunications to biosensing in the visible and infrared wavelengths [1]. In this context, large-scale photonic integrated circuits (PICs) operating in the ultraviolet wavelength range have received much less attention despite their potential impact in the fields of process analytical technologies, microscopy, biosensing, and photochemistry. Achieving sufficiently low propagation losses for relevant applications is one of the main challenge of UV PICs today. We have successfully tackled this challenge at a wavelength of $\lambda = 360\,nm$ [2] as discussed here by implementing label-free super-resolved structured illumination microscopy with a UV PIC.

Structured illumination microscopy (SIM) has received much attention in biology in recent years due to its scanning-free super-resolution property and its use of standard fluorescent labels [3]. Up to now, the SIM technique has mainly been investigated with illumination in the visible range due to the lack of high-transmittance microscopy objectives and ways of beam manipulation at UV wavelengths. Besides, current SIM microscopes are bulky and expensive due to the requirement of complex free space optical systems. Here, we have used UV-compatible PICs as an add-on module of a standard wide field microscope in order to implement SIM. Our approach not only boosts the performance of conventional microscopes in terms of optical resolution but also conveniently extend the illuminating wavelength from visible to UV wavelengths. The UV-PICs are designed to generate, switch on and off, and spatially shift several UV far-field fringed patterns that illuminate the fluorophores

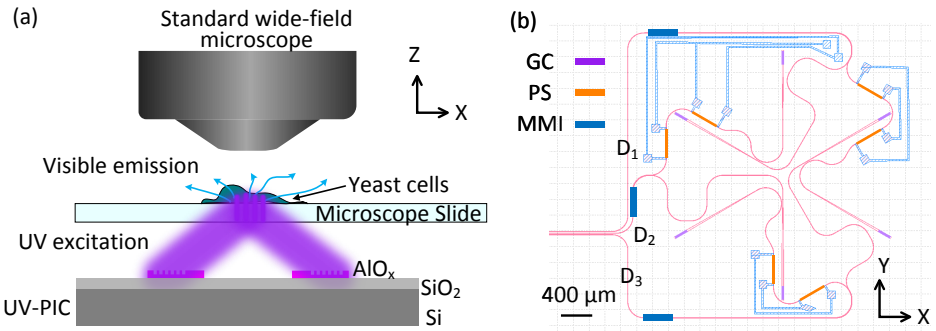

Fig. 1. (a) Schematic of the principle of structured illumination microscopy making use of UV-compatible photonic integrated circuits (b) Typical layout of the photonic integrated circuits. GC: grating coupler, PS: phase shifter, MMI: multi-mode interferometer.

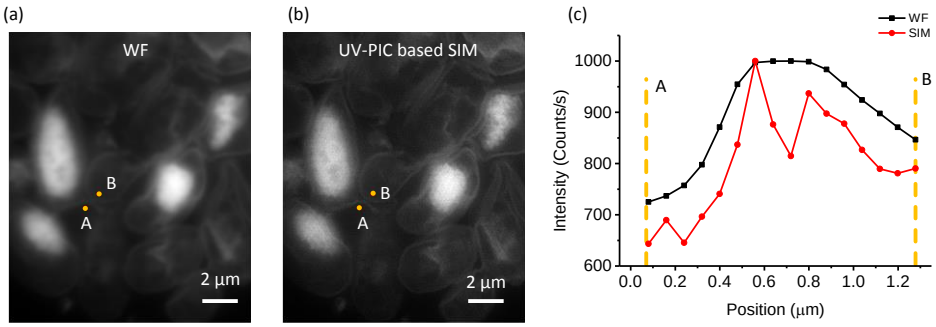

Fig. 2. (a) Optical images of the autofluorescence of NADH molecules in yeast cells using conventional microscopy, (b) UV-PIC-based SIM technique. (c) Intensity profiles of the segment A-B indicated in (a) and (b).

in the sample to be imaged.

2. Results

As shown in figure 1(a), the label-free sample prepared on a standard microscope slide is placed at the imaging plane of a wide-field microscope objective and excited by the UV structured illumination generated by the UV-PIC chip positioned below the sample. The photonic circuits are made of an aluminum oxide (AlO_x) layer on a SiO_2/Si substrate. The AlO_x layer grown by atomic layer deposition (ALD) exhibits good optical transparency at UV wavelengths down to $250\,nm$ with the reported propagation loss of $4\,dB/cm$ [4]. Figure 1(b) shows the layout of the designed photonic circuits which consists of multi-mode interferometers(MMI), grating out-coupler (GC), and thermal phase shifters (PS). In this experiment, low-loss single-mode waveguides of $3\,dB/cm$ connects the optical components operating at $\lambda = 360\,nm$. The waveguides are formed by fully etching a $120\,nm$-thick AlO_x layer, while the gratings are shallow etched with a etch-depth of $30\,nm$. Three pairs of grating out-couplers diffract one after the other the UV light from the on-chip waveguides into free space to generate three illuminating fringe patterns rotated by 120 degrees in the plane of the sample. Three different spatial shifts of each fringe patterns are achieved by tuning integrated thermal phase shifters. As a result, the photonic chip allows us to record a total of nine low-resolution images that are necessary to reconstruct the super-resolved SIM image.

Many intrinsic bio-molecules exhibit autofluorescence at visible wavelengths when excited with UV light, such as for instance nicotinamide adenine dinucleotide (NADH). Yeast cells are used in this experiment to validate the UV-PIC-based SIM technique. In this measurement, the excitation numerical aperture NA_{ex} of 0.5 and the collection NA_{co} of 1.32 are determined by the grating out-couplers and the microscope objective, respectively. A UV laser operating at a wavelength of $360\,nm$ is coupled from on optical fiber to the photonic integrated circuit. The autofluorescence of the NADH molecules in yeast cells exhibits a peak wavelength of $450\,nm$ in the emission spectrum. The distribution of the NADH molecules can be revealed by conventional wide-field (WF) microscopy and UV-PIC SIM microscopy, as shown in Fig. 2(a) and (b)) respectively. The intensity profile of the segment A-B in Fig. 2(a) and (c) is plotted in Fig. 2(c). It can be clearly seen that SIM microscopy is able to resolve more fine features than standard wide-field microscopy.

3. Conclusion

To conclude, we have successfully implemented an UV-PIC operating at $\lambda = 360\,nm$ for advanced super-resolved microscopy technique by imaging the autofluorescence of yeast cells in a low-cost, compact, and robust way. This work validates the relevance of the alumina-on-silica platform for UV photonic integrated circuits, which is compatible with large-scale fabrications.

References

1. W. Bogaerts, et al., "Programmable photonic circuits", Nature **586**, 207-216 (2020).
2. C. Lin, et al., "UV photonic integrated circuits for far-field structured illumination autofluorescence microscopy", Nature Communications **13**, 4360 (2022).
3. M. G. L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy", J. Micro. **198**, 82-87 (2000).
4. M. M. Aslan, et al., "Low-Loss Optical Waveguides for the Near Ultra-Violet and Visible Spectral Regions with Al2O3 Thin Films from Atomic Layer Deposition", Thin Solid Films **518**, 4935-4940 (2010).