Development of high index contrast waveguide platform for fluorescence based optical nanoscopy

Firehun Tsige Dullo[1], Øystein Ivar Helle[1], David Andre Coucheron[1], Marcel Lahrberg[1], Jean-Claude Tinguely[1] and Balpreet Singh Ahluwalia[1]*

[1] Department of Physics and Technology, UiT, The Arctic University of Norway, Klokkargådsbakken N-9019, Tromsø, Norway

e-mail: balpreet.singh.ahluwalia@uit.no

ABSTRACT

High-index contrast (HIC) based photonics integrated circuit platforms have played a vital role in integrating complex optical components in a small footprint. Here, we report chip-based nanoscopy (CbN) by using HIC waveguide platforms. In CbN, the waveguide chips generate, deliver and steer the laser illumination pattern. By using a nanoscopy technique of single molecule localization, we demonstrate CbN with a resolution of 50 nm. Furthermore, we show that chip-based structured illumination microscopy can overcome the 2× resolution enhancement set by the objective launch in conventional linear SIM.

Keywords: High index contrast, Silicon nitride, optical nanoscopy, super-resolution microscopy.

1. INTRODUCTION

Photonics integrated circuit (PIC) platforms have played a central role in miniaturizing and integrating complex optical components in a small footprint [1]-[5]. Among previously reported works on PIC platforms, those that are based on high index contrast (HIC) have gained the most attention. HIC waveguides have strong confinement of the light inside the waveguide because of the high refractive index contrast between the waveguide and the cladding material. HIC material also allows fabricating of optical functions in much smaller footprint, due to tight bend radii. Tight confinement of light inside HIC waveguide also enables fabrication of thin (e.g. 100 -100 nm thickness) optical waveguide enhancing the intensity of the evanescent field at the surface of the waveguide. Silicon on insulator (SOI) photonics waveguide platform, which is HIC waveguide platform, is a well-established technology. However, this technology is limited since silicon is only transparent above 1.1µm. Silicon nitride (Si_3N_4) which is transparent between 0.4 and 5 µm is widely used instead of silicon for a visible and near infrared wavelength range. This is particularly relevant for Raman spectroscopy, fluorescence spectroscopy and chip based super resolution microscopy.

Fluorescence microscopy has had a major impact on biomedical research, however, is bounded with the diffraction limit barrier inherent in optical microscopy [6]-[9]. Recent innovations have led to the development of super resolution microscopy also known as optical nanoscopy that allows for observation of fluorescent samples at resolutions below the diffraction limit. The methods of optical nanoscopy include techniques such as; the patterned light illumination techniques such as Stimulated Emission Depletion (STED) and *Structured Illumination Microscopy* (SIM) and localization-based techniques, which includes (direct) Stochastic Optical Reconstruction Microscopy ((d)STORM) and Photo-activation Localization Microscopy (PALM).

The conventional optical nanoscopy rely on using the same objective lens for both illumination and collection which results in a system complexity, small field of view, high-cost and slow imaging speed [1]. In this work, we report chip based nanoscopy (CbN) that can overcome these limitations.

2. Working Principle of Single molecule localization microscopy and structured illumination microscopy

Single molecule localization microscopy (SMLM): SMLM is a super-resolution imaging technique that utilises time-resolved localisation of switchable fluorophores, i.e. fluorophores that can be switched between a fluorescent “on”- and a dark “off”-state. Only a small and resolvable subset of all fluorophores attached to the sample is activated to the fluorescent “on”-state. As the signal originates from a single molecule, it is possible to find the position of each fluorophore with sub-resolution precision e.g. 20-50 nm by fitting 2-D Gaussian fit. After image acquisition the fluorophores are switched to the “off”-state (either using laser excitation or stochastical) and another subset is activated and imaged as mentioned before. Iteration of this process over several thousand images allows a multitude of fluorophores to be localised, whereupon the precise knowledge of their positions is used for the reconstruction of a super-resolved image. For performing SMLM in direct stochastic optical resonctruction microscopy (*d*STORM) mode, high intensity in the evanescent field is required, in the orders og 1-10KW/cm^2.

Structured illumination microscopy (SIM) works by using a patterned illumination to excite the sample. The overlap between the high frequency of the sub-diffraction details within the sample and the high frequency of the illumination pattern creates Moiré patterns, a pattern of lower frequency which is well collected by the objective. These Moiré patterns contain high frequency spatial information that would not be interpretable through diffraction-limited imaging. The pattern position and orientation is changed; to untangle the high frequency and isotropic resolution, respectively, and the emitted fluorescence signal for each position is recorded. Here, we report that creating the patterned illumination on a photonics chip enhances the resolution set by the objective launch in conventional linear SIM (λ/4N.A.) because photonic waveguide can create finer illumination pattern than the one set by the state-of-the-art objective lens since photonics waveguide gives better N.A. Furthermore, using photonics waveguide miniaturizing and integrating complex optical components.

3. Experimental Set-up and Results

In CbN, the photonics chip is used both for holding the sample under investigation (replacing the conventional glass-slide/coverslip), and for generating the required illumination needed to acquire super-resolved images [3]-[4], such as in SMLM and SIM. An experimental set-up for chip based nanoscopy is shown in a Fig. 1 a. A laser light is coupled into a waveguide chip by using objective lens or optical fiber. The samples are illuminated using the evanescent field present on top of the waveguide surface. The emitted fluorescence signal is collected using an upright microscope, fitted with an objective lens. Silicon nitride (Si_3N_4) based photonics waveguide platform for fluorescence nanoscopy is thoroughly investigated and opted [4]. The use of high-refractive index material allows to generate high intensity in the evanescent field, enabling the blinking (switch on and off) of the fluorophores: a key prerequisite for localization microscopy. The waveguide was made of 1-1000 µm wide and of thickness of 150 nm. The use of wide waveguides are essential to illuminate large group of cells (See Fig. 1c), while thin waveguides enable to enhance the surface intensity of the evanescent field.

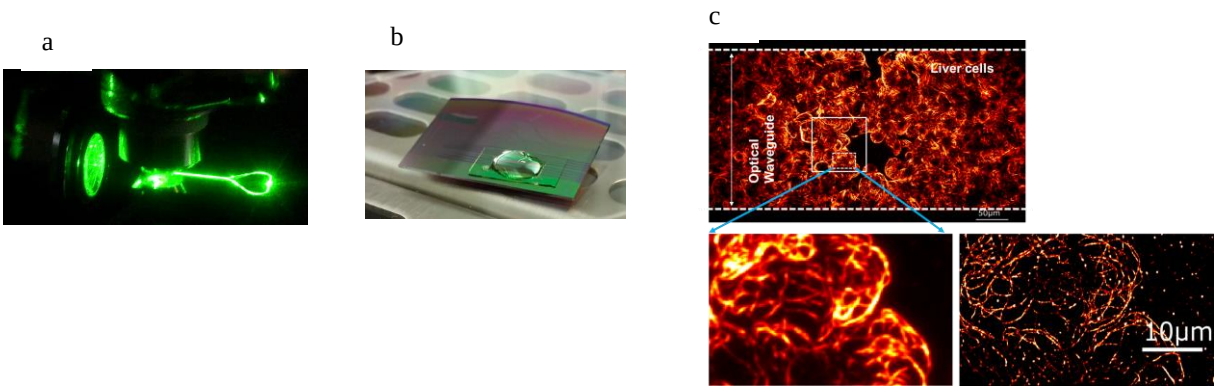

Fig.1a) Schematic diagram of chip-based nanoscopy, b) optical waveguide with a PDMS chamber;c) Chip-based TIRF and (d) chip-based dSTORM of liver sinusoidal endothelial cells (LSECs).

4. CONCLUSIONS

High-index contrast (HIC) based photonics integrated circuit platforms have played a vital role in integrating complex optical components in a small footprint. It has been shown that high surface intensity on top of HIC wavegudie platform can be exploited to perform super-resolution optical microscopy. In CbSIM, photonics chips are used to generate the illumination pattern needed for different optical nanoscopy methods. Furthermore, use of photonics waveguide miniaturizes complex optical microscopy which is presently based on free-space bulk optical components.

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