# Nanoscopy-on-a-chip: high-throughput super-resolution imaging

*(Invited paper)*

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## ABSTRACT

Fluorescence based super-resolution optical microscopy, commonly referred to as optical nanoscopy, enables visualization of nanoscale biological events and structures in living cells. Although optical nanoscopy offers unprecedented opportunities, the widespread adoption of nanoscopy is presently hindered by system-complexity, high-cost, lack of multi-modality and slow imaging speed of present nanoscopy techniques.

Here, we report chip-based nanoscopy (CbN). To achieve super-resolution, our main idea consists in taking the generation, the steering and the delivery of the laser illumination pattern from the microscope and transferring it to the photonic chips. Using the principle of single molecule localization, we demonstrate CbN with a resolution of 50 nm. Furthermore, wedemonstrate the capability of CbN to acquire super-resolved images over millimetre field-of-view scale; a 100-fold increase in imaging area as compared to other nanoscopy solutions.

The waveguide platform has been developed using high-refractive index material such as silicon nitride ($Si_3N_4$) and tantalum pentoxide ($Ta_2O_5$). High-index contrast materials enable tight confinement of the light inside the waveguide, allowing for ultra-compact waveguide structures with small footprint benefitting from high intensity in the evanescent field.

**Keywords**: Optical nanoscopy, super-resolution microscopy, bio-imaging, silicon nitride, tantalum pentoxide

## 1. INTRODUCTION

Fluorescence based optical microscopy has emerged as a vital tool in modern bio-medical imaging and diagnosis[1-3]. Different labelling strategies allow high specificity towards the enabling fluorescence microscopes to generate images with excellent signal to noise ratio and high specificity. However, the optical resolution of the fluorescence microscopy is limited due to the diffraction limit, i.e. around 200-250 nm and 500 nm along the lateral and the axial directions, respectively.

During the last decade, there has been a significant growth in the field of super-resolution optical microscopy [1-3] to image targets with resolution below the diffraction limit. The field is referred to as optical nanoscopy. Optical nanoscopy has revolutionized bio-imaging and provided insights into structures and phenomena that were previously only accessible with electron microscopy on fixed specimens. The methods of optical nanoscopy include techniques such as structured illumination microscopy (SIM), stimulated emission depletion microscopy (STED); single molecule localization microscopy (SMLM) techniques such as photo-activated localization microscopy (PALM), and (*direct*) stochastic optical reconstruction microscopy ((*d*)STORM); and fluctuation based optical nanoscopy techniques such as super-resolution optical fluctuation imaging (SOFI), multiple signal classification algorithm (MUSICAL), and entropy-based super-resolution imaging (ESI).

SMLM and STED provide the best resolution (20-50 nm), but they are slow and require special dyes. SIM is a wide-field technique, thus faster, works with common dyes, and is suitable for imaging of live cells. However, SIM provides optical resolution only upto 100 nm with a practical speed is in the range of 0.1-1 Hz, which is much slower than the imaging speed of commonly used diffraction-limited live cell microscopy (up to 300 Hz).

**Chip-based nanoscopy (CbN):** In optical nanoscopy, the illumination path is as important as the detection path in order to provide images of high quality. One of the main tricks behind breaking the optical diffraction limit is to tailor the laser illumination pattern and emission collection. Conventional optical nanoscopy techniques use a complex microscope for imaging and a simple glass slide solely to hold the sample. Here, we report the inverse configuration: a complex but mass-producible photonic chip (optical waveguides) to both hold the sample and generate and deliver the illumination pattern needed to acquire super-resolved images, and a standard optical microscope to acquire a super-resolved image.

## 2. Development of the waveguide chip platform

The waveguide platform should be optimized for the visible wavelengths (405-780 nm) for optical nanoscopy. It is desirable to use a material having low propagation losses**,** low absorption and low auto-fluorescence at visible wavelengths. The *d*STORM [3] rely on most of the fluorophores to be forced into long-lived dark states, where

only sub-sets are allowed to return to the fluorescent state resulting in blinking of the fluorescent molecules. When the blinking is sufficiently sparse, a high-resolution image can be built up over several thousand individual sub-sets (individual images). The *d*STORM technique requires a high intensity evanescent field of around 1-10 kW/cm$^2$. The high intensity is required to enable blinking of the fluorophores, a key requirement in SMLM. We employ thin (150-200 nm) optical waveguide made of high-refractive index contrast (HIC) materials such as silicon nitride ($Si_3N_4$) [4] and tantalum pentoxide ($Ta_2O_5$) [5] to achieve such high power intensities. For such a waveguide, up to 10-15% of the guided power can be then made available in the evanescent field.

When single mode conditions are required, we employed shallow rib geometry, i.e. 2-4 nm etched rib heights out of a total height of 150 nm [4]. Multimode waveguides do not directly provide homogeneous illumination on the surface due of interference between several guided modes inside the guiding layer. However, by oscillating the input-coupling beam combined with averaging the acquired image stacks helped to generate acceptable homogeneous illumination (around 15% intensity modulation). Strip geometry was employed in this work, with a waveguide width of 100-250 µm and total thickness of 150-220 nm.

**3. Super-resolution imaging using chip-based nanoscopy platform**

In chip-based nanoscopy, the generation of the illumination light (evanescent field) is independent of the collection objective lens, which gives several advantages compared over other nanoscopy systems. The experimental-setup is compact and stable; pre-aligned for easy multi-color imaging without additional optical alignment and mechanical adjustments; and simple changing of the magnification/imaging objective lens without influencing the illumination.

Figure 1 shows the experimental set-up that consists of an upright microscope, laser coupling unit and the photonic chip. The photonic chip is used as a substrate, and the laser light is coupled into the waveguide either through an objective lens or by using optical fiber. The cells are illuminated using the evanescent field present on top of the waveguide surface. The fluorescence light is collected using an upright microscope, fitted with several objective lenses of different magnification.

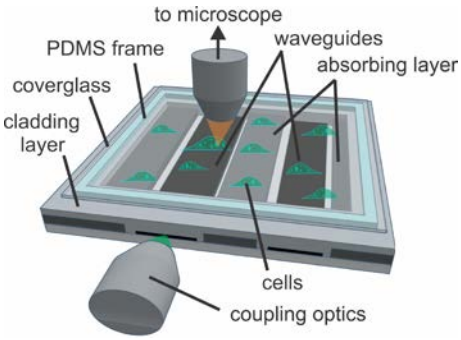


*Figure 1 Experimental set-up of chip-based optical nanoscopy. The sample is placed directly on top of waveguide and is illuminated by the evanescent field. The optical waveguide is made of high refractive index contrast material. The fluorescence light from the waveguide surface is collected by an upright microscope.*

The evanescent field decays exponentially from the waveguide surface, illuminating only a thin section of the cells (around 150 nm) close to the waveguide surface. The illumination from the waveguide-chip therefore enables total internal reflection fluorescence (TIRF) microscopy. Images acquired using chip-based TIRF microscopy are shown in Figure 2. Liver sinusoidal endothelial cells (LSECs) were seeded directly on top of the photonic-chips and stained with fluorescent dyes. The cells were fixed on the chips and first imaged at low magnification (4x) to locate isolated specimens and to find regions of interest (top image of Figure 2A). Once the region of interest was found, imaging of the fluorophores was done with a higher resolution objective at three different wavelengths, 488 nm, 561 nm and 660 nm (cell membrane in green, actin in blue, and proteins undergoing endocytosis in red – bottom image of Figure 2B). The chip-based TIRF microscopy decouples the illumination and the collection light path, allowing changing the magnification of the imaging objective lens without altering the TIRF illumination generated by the optical waveguide, as shown in Figure 2. The optical waveguide platform supports multiplexing, i.e. generation of TIRF illumination for different wavelengths without the need of additional optical components or adjustments to the set-up.

Figure 3 shows chip-based nanoscopy using *d*STORM principle [6] on LSECs. Figure 3a shows diffraction-limited TIRF image and Figure 3b shows *d*STORM image, both acquired using the waveguide-chip platform. The LSECs possess a unique structure on its membrane, nano-sized holes (50-200 nm) commonly referred to as fenestrations. The size of fenestration is below the diffraction limit (200 nm). The fenestration is therefore not visualized using diffraction limited TIRF microscopy but can be imaged using chip-based *d*STORM. A high numerical aperture and high magnification objective lens (60x/NA1.2) is used in Figure 3. As the evanescent

field is present over the entire surface of the optical waveguide, TIRF illumination can be achieved over large surface areas by simply using a wide optical waveguide (e.g. 200-500 µm). Recently, chip-based *d*STORM using a 20x/NA0.45 objective lens was shown capable of imaging field-of-view of 0.5x0.5 $mm^2$ covering more than 50 cells with a resolution of 140 nm [6].

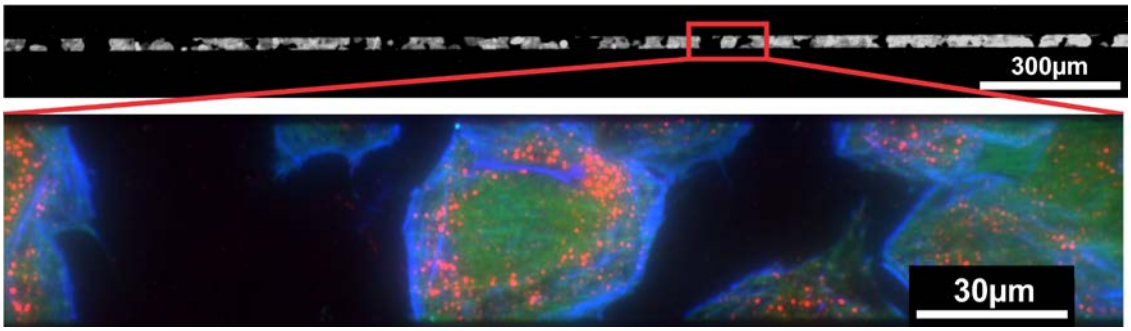


*Figure 2 Chip-based TIRF microscopy of liver sinusoidal endothelial cells (LSECs). The top image is acquired using an objective lens with 4X magnification and bottom image is acquired using an objective lens with 60X magnification. A low magnification objective lens is used to scan large areas. As evanescent field is generated by the waveguide, the imaging objective lens can simply be changed without altering the illumination light path. Staining represents: plasma membrane (in green), actin (in blue), and an endocytosed protein (in red)*

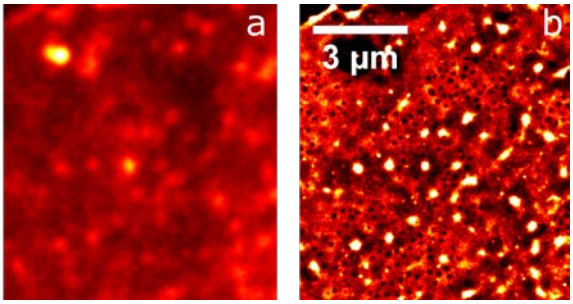


*Figure 3 a) Chip-based TIRF and (b) chip-based dSTORM of liver sinusoidal endothelial cells (LSECs).*

## 4. CONCLUSIONS

The Photonic-chip offers great miniaturization of versatile sets of design to shape and control optical beams that can be barely engineered with conventional far-field optics. Development of nanoscopy using waveguide chips opens a new research activity in bio-imaging, as the chip-platform can easily be integrated (retrofit) into conventional microscopes. In addition, CbN can enable integration of multiple nanoscopy modalities onto a single waveguide-chip using a standard microscope. The compatibility of CbN with optical fibre components will enable easy wavelength multiplexing and an alignment-free illumination light path. In addition CbN is shown to surpass the technical capabilities of present day optical nanoscopy in terms of large field-of-view, high-throughput imaging and simplicity. Development of on-chip TIRF microscopy and optical nanoscopy is flexible towards future integration with other integrated photonic-chip based functions such as on-chip sensing, spectroscopy [7], and particle tracking and trapping [8].

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