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On-chip Structured Illumination Microscopy Enabled by Femtosecond Laser Engineered Microscope Slide

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Abstract: We present a compact glass slide engineered via femtosecond laser micromachining with the integration of optical waveguides, micro-optics and thermal shifters. The device generates dynamic patterns enabling structured illumination microscopy on conventional fluorescence microscopes. © 2025 The Author(s)

1. Introduction

Optical nanoscopy has revolutionized fluorescence microscopy by surpassing the classical diffraction limit [1], as demonstrated by the many developed super-resolution techniques as STED, STORM, and PALM. Among them, Structured Illumination Microscopy (SIM) is attractive because of its high-resolution imaging, low phototoxicity, and, differently from the other techniques, its compatibility with common fluorescent labeling [2, 3]. SIM is based on the use of patterned excitation, typically achieved via laser beam interference. In its original implementation, diffraction gratings and mechanical stages controlled the pattern. Recent advances use spatial light modulators for precise phase modulation, but bulk optical setups remain complex and require frequent adjustments. Alternative approaches, such as fiber-based systems [4] and photonic chips [5], address these issues but still face challenges like free-space optics requirements or high coupling losses. Glass-based photonic devices offer a promising solution. Femtosecond Laser Irradiation followed by Chemical Etching (FLICE) [6] enables precise fabrication of optical waveguides with controlled birefringence and allows the integration of thermal phase shifters with fast switching times, supporting high-speed applications. Recently, a hybrid fiber-chip pattern generator [7] has demonstrated its effectiveness as a SIM light source, achieving super-resolution imaging in widefield microscopes. In conventional SIM, the objective lens is used for both illumination and fluorescence collection, limiting resolution enhancement to a factor of two. A recent method [8] decouples these optical paths, slightly exceeding this limit while maintaining a large field of view. Here, we propose an integrated approach to this concept by developing a fully integrated structured illumination pattern generator directly on an engineered microscope slide. This innovation will allow conventional widefield microscopes to be easily upgraded to SIM microscopes in a reconfigurable and cost-effective manner. The engineered slide will incorporate all necessary optical components to generate a permanently aligned structured illumination area. Simply placing it as a cap on the sample coverslip will enable structured light illumination, eliminating the need for complex optical alignment. The device is also compatible with 3D SIM implementation. This easy-to-use platform has the potential to enhance the accessibility to super-resolution microscopy by reducing costs and expanding accessibility, thereby fostering widespread adoption in biological research and morphological analysis applications.

2. Engineered microscope slide for SIM

1.1. Device design

The engineered slide was designed using a 60 × 15 × 1 mm^3 fused silica substrate, compatible with standard fluorescence microscopes and the schematic layout is shown in Fig. 1. The key components integrated into the slide include: an optical circuit (tritter) in which a cascade of evanescent directional couplers splits incoming light into three equal parts, a mirror sequence to redirect the light composed of flat mirrors that reflect the split beams toward collimating mirrors. The latter, positioned at specific angles, direct the beams onto the sample, creating the structured illumination pattern. Thermal phase shifters, positioned over two of the three optical paths, allow dynamic pattern adjustments without mechanical movement.

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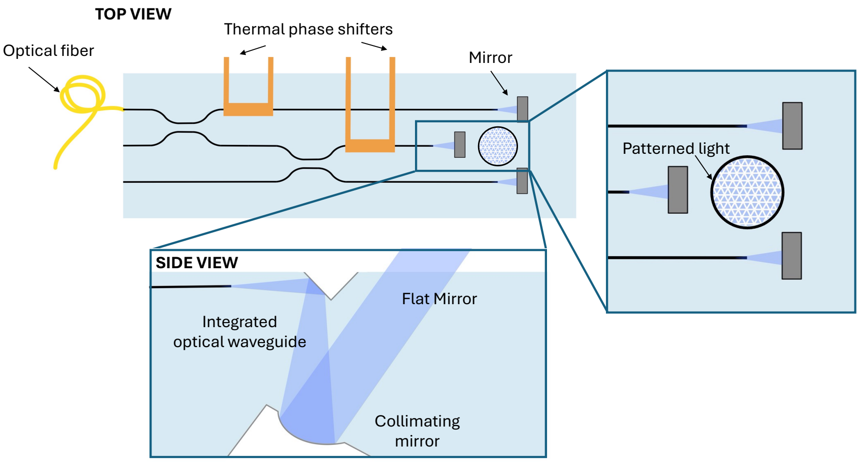

Fig. 1. Schematic top and side view of the integrated pattern generator.

1.2. *Engineered microscope slide fabrication*

Three distinct pattern generators were implemented on a single slide, each producing different spatial frequencies by varying the tilt of the collimating mirrors. This configuration enables adaptability to different microscope objectives, ensuring optimal pattern periodicity for various numerical apertures.

Mirrors

Both flat and collimating mirrors were fabricated using FLICE. The femtosecond laser irradiation induces nanogratings in the silica, enhancing chemical etching efficiency. The engineering of the irradiation geometry allows achieving the desired shape of the microoptics. The mirrors were then polished using CO_2 laser processing to improve surface smoothness before being coated with a 250 nm aluminum layer, achieving 87% reflectivity at 488 nm.

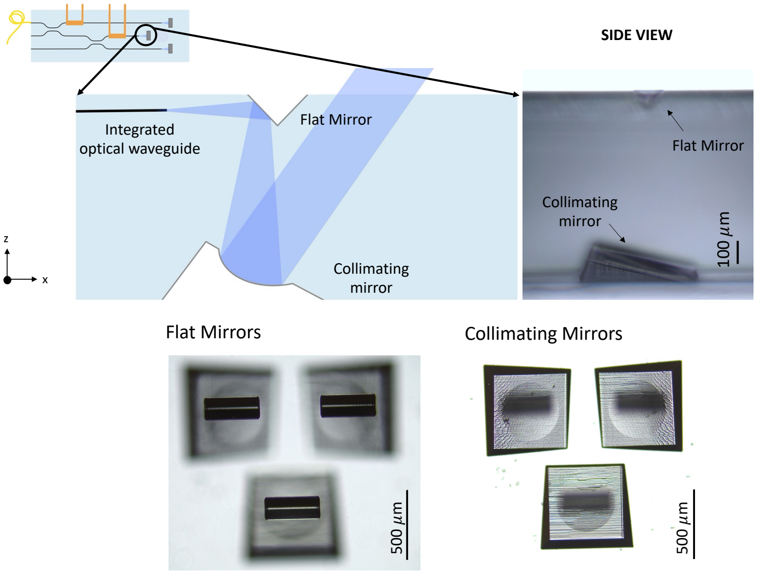

Fig. 2. Side and top view of the flat and collimating mirror fabricated as in the reported scheme of the final device..

Flat mirrors were structured with a triangular cross-section to enhance uniform metal deposition, while collimating mirrors were designed using optical simulations, pre-compensated for the etching process, to achieve precise beam collimation.

Optical Circuit

The optical waveguides composing the tritter were also fabricated using FLM. Waveguides were optimized for low propagation losses, and curved waveguides with minimal bending losses were implemented to create compact circuits. Good results were found irradiating the sample at 515 nm, 1 MHz repetition rate with a multiscan geometry. Propagation losses of about 1.72 dB/cm were achieved at an operating wavelength of 488 nm. Directional couplers, designed for efficient beam splitting, were fine-tuned to achieve a 33%-66% and 50%-50% power distribution. Starting from the optimal values of splitting ratio found for the couplers, the tritter fabrication and characterization was carried out. A tritter suitable for the engineered glass slide was found with an interaction length of 0.29 mm for the 33%-66% and 0.38 mm for the 50%-50% directional couplers, obtaining a reasonable approximation of an even power splitting with a measured output power distribution of 30.5%, 28.20% and 41% in the three arms.

Device assembly

The tritter was integrated into the final chip, ensuring precise three-way beam splitting. The three beams exiting the tritter generate an interference pattern on the glass surface. Additionally, thermal phase shifters were fabricated by selectively ablating a 100 nm gold layer deposited on the chip. The resistive elements modulate the structured pattern dynamically, allowing SIM imaging with only seven acquired frames. A picture of the final device is shown in Fig.3 along with an image of the generated hexagonal pattern.

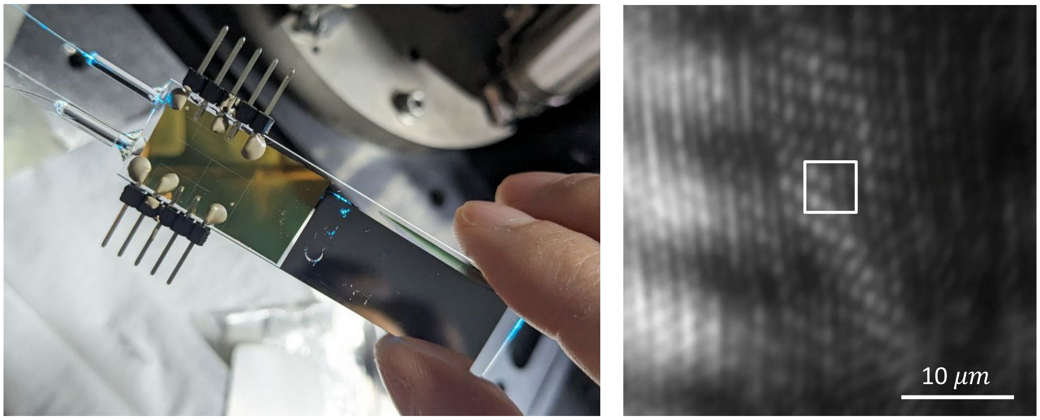

Fig. 3. (Left) Assembled device: optical fibers are pigtailed to the optical circuit with a tritter and gold thermal shifters. The blue light coupled out of the waveguides impinges on the mirrors in the right part of the engineered glass. (Right) Modulated light obtained by the interference of the three beams; in the white square the hexagonal pattern is highlighted.

3. References

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