Microelectronic Engineering 195 (2018) 41–49

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

Research paper

Focused ion beam nanomachining of tapered optical fibers for patterned light delivery

Filippo Pisano[a,*,1], Marco Pisanello[a,1], Leonardo Sileo[a], Antonio Qualtieri[a], Bernardo L. Sabatini[b], Massimo De Vittorio[a,c,2], Ferruccio Pisanello[a,*,2]

[a] Istituto Italiano di Tecnologia, Center for Biomolecular Nanotechnologies, 73010, Arnesano, Lecce, Italy
[b] Dipartimento di Ingegneria dell'Innovazione, Università del Salento, 73100 Lecce, Italy
[c] Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School, 02115, MA, Boston, USA

ARTICLE INFO

Keywords:
Focused ion beam
Milling
Optical fibers
Optogenetics
Nanomachining

ABSTRACT

With the advent of optogenetic techniques, a major need for precise and versatile light-delivery techniques has arisen from the neuroscience community. Driven by this demand, research on innovative illuminating devices has opened previously inaccessible experimental paths. However, tailoring light delivery to functionally and anatomically diverse brain structures still remains a challenging task. We progressed in this endeavor by micro-structuring metal-coated tapered optical fibers and exploiting the resulting mode-division multiplexing/de-multiplexing properties. To do this, a non-conventional Focused Ion Beam (FIB) milling method was developed in order to pattern the non-planar surface of the taper around the full 360°, by equipping the FIB chamber with a micromanipulation system. This led us to develop three novel typologies of micro-structured illuminating tools: *(a)* a tapered fiber that emits light from a narrow slot of adjustable length; *(b)* a tapered fiber that emits light from four independently addressable optical windows; *(c)* a tapered fiber that emits light from an annular aperture with 360° symmetry. The result is a versatile technology enabling reconfigurable light-delivery that can be tailored to specific experimental needs.

1. Introduction

The use of optogenetics to trigger neural activity has allowed for a fascinating set of new neuroscience studies [1,2], mostly made possible by the cell-type specificity of the genetic codification of microbial opsins [3,4]. Together with the improvement in targeting specific neural populations, technologies to deliver light into brain tissue have seen important advances in the last few years [5–7], as innovative approaches offered promising alternative options to the illumination with flat cleaved optical fibers [4]. In fact, despite offering practical advantages such as freedom of choice in the operating wavelength, substantial optical output power and ease of use, the traditional flat fiber illumination is affected by relevant drawbacks. For instance, a flat fiber interfaces with a limited area in the vicinity of its tip. This, in turn, poses strict requirements to the surgery procedure in order to implant the fiber tip, and a recording probe, in the desired position. These shortcomings have been overtaken by multifunctional optical fibers that enabled researchers to deliver light and drugs in the same brain region and to simultaneously record extracellular electrical activity [8]. This allowed the co-localization of viral vectors and light delivery, improving the efficiency of the optical control and reducing the invasiveness of the procedure [9]. At the same time, tapered optical fibers (TFs) were proposed as a tool that can dynamically illuminate both large and spatially confined volumes at multiple wavelengths [10–12]. On the other hand, despite being limited to a single wavelength, μLED probes paved the way to high-resolution, multi-site optogenetic stimulation and recording of neural activity with low thermal stress [13–17]. In addition, wireless probes with multiple integrated μLEDs, recording electrodes and drug delivery capabilities have been employed in the development of battery-free systems designed for freely behaving experiments [9,18–20].

An open question in the field is, however, how to tailor light delivery patterns with the very diverse functional and anatomical structure of different brain regions. In this respect, all the above-mentioned techniques have important limitations. Polymeric fibers can deliver light only from an invasive flat-cleaved facet (whose implant cross

* Corresponding authors.
E-mail addresses: filippo.pisano@iit.it (F. Pisano), ferruccio.pisanello@iit.it (F. Pisanello).
[1] These authors equally contributed to this work.
[2] These authors equally contributed to this work.

https://doi.org/10.1016/j.mee.2018.03.023
Received 18 January 2018; Received in revised form 13 March 2018; Accepted 26 March 2018
Available online 28 March 2018

section diameter is in the order of hundreds of micrometers); miniaturized microscopes allow for high resolution in vivo, but suffer from a limited imaging depth; μLEDs cannot be used for long term stimulation due to generated tissue heating; tapered optical fibers have a cylindrically-symmetric emission that is hard to break.

We recently proposed a method that exploits mode division multiplexing and de-multiplexing in TFs to switch light-delivery between different regions of the brain along a millimeters-long taper [10,11]. This was made possible by a specific optical property of the tapered region: when the waveguide narrows, the number of guided modes sustained by the fiber decreases and light is gradually leaked along the taper. By selecting the subset of injected modes in the waveguide, this light leakage can be controlled and exploited to generate site-selective and dynamically-addressable light delivery up to about two millimeters deep in the mouse brain [10]. These properties are preserved [23,24] when the light guiding mechanisms into the waveguide is modified from pure dielectric confinement to metallic confinement by depositing a metal layer all around the taper [25,26]. However, an important difference can be traced between metallic and dielectric confinement in TFs. While in the dielectric case light is gradually leaked from the tapered section, in the metallic case non-leaked light keeps propagating in the narrowing taper, with an increasing transversal component of the wave vector (k_t).

Leveraging on this effect, this work exploits the availability of high k_t values in high numerical aperture metal-coated TFs to realize patterned light emission from the taper surface over taper segments that extend over 2 mm. Using focused ion beam (FIB) milling, the metal coating was selectively removed following specific geometries along and around the taper, including long slots, multiple square windows and annular apertures with 360° symmetry. The annular aperture was obtained by implementing an unconventional approach to FIB milling, with the fiber being roto-translated during the process, that represents, to the best of our knowledge, the first method to structure a tapered fiber all-around the taper surface with the high resolution allowed by FIB. For the multiple square window device, induced thermal effects due to evanescent light within the taper were measured, excluding significant temperature increase on the taper surface when outcoupling an optical density < 60 mW/mm^2.

These patterns of light emission can be dynamically controlled by selecting the subset of guided modes injected into the waveguide. The result is a technology that allows adapting light delivery geometry tailored to specific experimental needs, introducing an additional degree of freedom on illumination methods for optogenetic control of neural activity.

2. Material and methods

2.1. Device fabrication

Tapered, metal-coated and patterned fibers were realized from NA = 0.39 and NA = 0.66 fibers with core/cladding diameters of 200 μm/225 μm and 200 μm/230 μm respectively, following the procedure described in detail by Sileo et al. [27]. Tapers with different taper angle ψ (see Fig. 1(a) for definition) were obtained from OptogeniX (OptogeniX s.r.l., Arnesano, Italy, www.optogenix.com). A 600 to 800 nm-thick layer of aluminum was deposited on TFs by means of thermal evaporation. Aluminum was preferred with respect to gold by virtue of its higher reflectivity at the absorption peak of ChR2. However, it is important to mention that in case of chronic employment of the aluminum-coated TFs, the taper should be encapsulated within a water proof material such as Parylene-C, in order to prevent tissue inflammation. During the evaporation process, TFs were rotated around their axis at a constant angular speed of a few rpms using a stepper motor [27]. Optical apertures were realized on the metal coating through FIB milling using a FEI Helios Nanolab 600i Dual Beam system, with ion beam currents of I_{FIB} = 9.3 nA or I_{FIB} = 2.5 nA (corresponding to a beam diameter of 249 nm and 133 nm, respectively), dwell time of 1 μs, and pixel pitch of 124.5 nm [25,27]. Dwell time and pixel pitch were kept constant in the realization of the different geometries, while I_{FIB} was modified to optimize the processing time with respect to the required resolution. Simultaneous SEM imaging was performed in order to stop the milling processes as soon as the fiber surface underneath the metal layer was completely exposed. Fig. 1(a) shows a schematic representation of the SEM-FIB chamber, with a definition of a reference frame used throughout the manuscript.

The ring-shaped window was realized using a micromanipulator (RoTip in conjunction with MM3A-EM, Kleindiek Nanotechnik GmbH) equipped with a custom holder to roto-translate the TF during the milling process. Successive millings were performed on rectangular 10 × 20 μm^2 windows, overlapping for ~1 μm along the shortest side (oriented perpendicularly to the fiber axis). The fiber was rotated by 20° around its axis between subsequent processes. The eccentricity of the micromanipulation system was corrected by translating the fiber to ensure that the milling happened over the TF portion facing the ion gun at the center of the field of view and in the ion beam focal plane. Data reported in this work were obtained from: four slot-emitting TFs (of which two 0.39 fibers with 500 μm slot, one fiber with two 250 μm slots on opposite sides and one 0.66 fiber with a 1.5 mm long slot); three four-window emitting fibers fabricated from 0.66 fibers; one 0.39 fiber with a ring-shaped window.

2.2. Light injection into the fiber

For full Numerical Aperture (NA) light injection, a continuous wave (CW) diode-pumped solid state (DPSS) laser source (λ = 473 nm, Laser Quantum Ciel 473, Laser Quantum, Stockport, UK) was collimated with a 10× beam expander and focused into the fiber with an objective lens (Olympus UIS-2 Plan N 40× NA = 0.65, Olympus Corp., Tokyo, Japan). For angle-selective light injection, light was coupled into the TFs by using the optical path depicted in Fig. 1(b). The CW beam was coupled to a patch fiber through a scanning system composed by lenses L_1 (focal length f_1 = 100 mm, Thorlabs LA1050-A, Thorlabs Inc., Newton, New Jersey, US) and L_2 (f_2 = 100 mm, AL50100-A), a galvanometric mirror GM (Sutter RESSCAN-MOM), and by lens L_3 (f_3 = 32 mm, AL4532-A). Lens L_1 focuses light on the rotation axis of the GM, while lenses L_2 and L_3 relay the beam deflected by the GM to the input facet of the fiber. Given a deflection θ_s imposed to the beam by the GM, the input angle θ into the optical fiber is given by $\theta = \tan^{-1}(f_2 \cdot f_3^{-1} \cdot \tan\theta_s)$. TFs were connected to the patch fibers of matching NA through a ferrule-to-ferrule butt coupling.

2.3. Light emission characterization

The tapered section of the fiber was immersed in a phosphate-buffered saline (PBS):fluorescein solution positioned in the sample plane of an epi-fluorescence upright microscope equipped with a 4× objective lens (Olympus XLFluor 4×/340). The 473 nm light emerging from the TF generated green fluorescence into the PBS:fluorescein droplet, which was collected by the objective, passed through a Fluorescein Isothiocyanate (FITC) filter and was imaged on a sCMOS (scientific grade Complementary Metal Oxide Semiconductor) sensor (Hamamatsu ORCA-Flash4.0 v2). Image acquisition was synchronized with the movement of the galvanometric mirror (Fig. 1b) using custom-written LabView software (National Instrument, Austin, Texas, US). Fluorescence intensity profiles were extracted from the acquired images along lines parallel to the taper surface.

2.4. Temperature measurement

The temperature increase of the four-window device during light delivery from each aperture was measured in air through a thermal Infra-Red (IR) camera (Testo model 875-1i, Testo Ltd. Alton,