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Perspectives of multimode fibers and digital holography for optogenetics

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ABSTRACT

Optogenetic approaches allow the activation or inhibition of genetically prescribed populations of neurons by light. In principle, optogenetics offers not only the ability to elucidate the functions of neural circuitry, but also new approaches to a treatment of neurodegenerative diseases and recovery of vision and auditory perception. Optogenetics already has revolutionized research in neuroscience. However, new methods for delivering light to three-dimensionally distributed structures e.g. in the brain are necessary. A major hurdle for focusing light through biological tissue is the occurring scattering and scrambling of the light. We demonstrate the correction of the scrambling in a multimode fiber by digital optical phase conjugation with a perspective for optogenetics.

Keywords: Measurement and control technology, spatial light modulators, digital camera techniques, neurophotonics

1. INTRODUCTION

Early after the invention of the laser it has been recognized that holography allows to reach the long-sought goal to see through turbid objects such as biological tissue and multimode fibers (MMF). In recent years, digital optoelectronic techniques for the correction of complex distortions of light have been developed, driven by the strong advancements of fast cameras, spatial light modulators (SLM) and signal processing techniques such as graphics processing units and field programmable gate arrays. Pioneering work was accomplished by Vellekoop and Mosk [1], who generated sculpted light in scattering media by an iterative optimization using an SLM. By maximizing the feedback signal, focusing through the turbid scattering media was demonstrated. The linear and deterministic light propagation through turbid scattering media is described by the complex transmission matrix between incident waves and outgoing waves. Measuring the matrix elements by digital holography and displaying the inverse matrix on an SLM allows to image through turbid media [2]. The time reversibility of the occurring scattering process is directly expressed in digital optical phase conjugation (DOPC) techniques. Using DOPC, seeing through chaos in diffuse media has been applied for an unscrambled transfer of spatial information intensively. To name are the focusing inside biological tissue, scanning of spots with deep penetration depth, the generation of complex patterns, endoscopic imaging, rotating of biological cells [3], and several further applications in biology and medicine [4]. However, the spatial information is transferred synchronously only. However, several applications require individually addressable signals such as in optogenetics, where e.g. multiple cells of a neuronal network need to be illuminated independently.

1.1 Optogenetics

Optogenetic approaches allow the activation and inhibition of genetically prescribed populations of neurons based on light-sensitive proteins, known as opsins, which mediate the conversion of a photon into an electrochemical signal [5-7]. Opsins occur naturally in many algae, bacteria, archaea and fungi. Injecting a harmless virus loaded with DNA from opsins such as channelrhodopsin2 (ChR2) from the unicellular alga *Chlamydomonas reinhardtii* to the brain of mammals results in light-sensitive neurons. Using blue light, ChR2 can be activated, which results in a transport of sodium cations through the ion channel of the cell membrane. Thus, a depolarization (positive charge) is yielded, hence an activation of the cell.

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A hyperpolarization (negative charge), hence an inhibition and silencing of the cell can be achieved by the stimulation of Halorhodopsin (NphR) from the archaea *natronomonas pharaonis* with yellow light. NphR is a light-driven chloride pump. Chloride anions will be transported through the ion channel. ChR2 and NphR can be easily expressed in neural cells of transgenic animals.

In conclusion, the conductivity, resistance and ion current of the channels can be modulated by the light. This can be considered as an “optical transistor”, since the light modulates the current through the membrane. The transport of ions across the cell membrane alters the electrical potential of the neurons and steers the release of the neurotransmitter from a storage vesicle into the synaptic cleft.

Historically viewed, the first experiments in optogenetics were conducted by Gero Miesenböck in the year 1999. The widely used optogenetic tools, based on the microbial rhodopsins, have been presented as directly light-gated ion channels by Peter Hegemann, Georg Nagel and Ernst Bamberg in the year 2002 [5]. To activate cells by light, and even to evoke coordinated behavior was pioneered by Ed Boyden and Karl Deisseroth in 2005 [6]. In 2007, NpHR was introduced, enabling together with ChR2 a light-activated push-pull control over membrane potential. Action potential firing and even coordinated behavior of transgenic animals were demonstrated [8]. The history of 10 years research of microbial opsins in neuroscience was reviewed recently [7]. Breakthrough Prizes in Life Sciences 2016 were awarded to Ed Boyden (Massachusetts Institute of Technology) and Karl Deisseroth (Stanford University and Howard Hughes Medical Institute) for the development and implementation of optogenetics – the programming of neurons to express light-activated ion channels and pumps, so that their electrical activity can be controlled by light.

Optogenetics offers not only the ability to elucidate the functions of neural circuitry, but also new approaches to a treatment of brain disorder. Optogenetics is hope for fighting against a large number of diseases of the central nervous system, such as autism spectrum disorders and anxiety disorder, fear, depression, attention deficit hyperactivity disorder, obsessive compulsive disorder, drug addiction, stroke, schizophrenia, chronic pain, Parkinson’s disease, epilepsy, as well as recovery of vision [9, 10] and auditory perception. For the alleviation of symptoms of Morbus Parkinson, which is a result of neurodegenerative processes, only two methods are in clinical use currently: Pharmacology and deep brain stimulation, using electrophysiology with stereotactic application of a metallic bipolar or quadrupole electrode to the nucleus subthalamicus. Both methods have significant unwanted side effects and single cell targeting is generally not achieved. By optogenetics, in contrast, virus induced transduction of cells with ChR2 allows the activation of the target structure in the brain with minimal invasive surgery, when using appropriate light sources. This is advantageous, since defined cell populations can be targeted by cell type-specific expression of the light-sensitive proteins, populations of cells can be addressed at once, the tissue needs to be penetrated only by light (not mechanically) and optogenetic neuronal activation and inhibition is highly reversible. A high temporal and spatial resolution in the range of microseconds and microns is achieved, respectively. It opens ways for the stimulation of substructures of the nucleus subthalamicus. The latter would give the chance to get a deeper understanding of the cause of this disease. Furthermore, optogenetic control of neurite growth has been explored for nerve regenerative therapies as a cell-specific alternative to electrical stimulation [11].

Optogenetics studies have been conducted in rodents and to some extent in primates using *in vivo* and *in vitro* acute slices. However, the translation to study human diseases is lacking. Recent advances in stem cell technology facilitate the generation of human neuronal cells and tissues from ethically unproblematic human induced pluripotent stem (iPS) cells [12]. There is a high interest in human disease modeling at the single cell level but also studying long-term potentiation (LTP), a form of synaptic plasticity, an elusive mechanism in brain physiology (see the Center for Regenerative Therapies Dresden of TU Dresden, principal investigator Volker Busskamp).

Various techniques have been used to deliver light into the brain of living mammals [13-16]: (1) Implanted μ LEDs are attractive for various biomedical application such as cochlea optogenetics and the treatment of neuropsychiatric disorders, including Parkinson’s disease and epilepsy. However, they generate substantial local heating [7] and the spatial resolution is usually too low for cellular stimulation. (2) Light delivery with an optical access through a cranial window involving skull removal or thinning for *in vivo* studies. One challenge is the multiple scattering of light inside the brain tissue. The effect of light scattering can be reduced for example by developing proteins which are light-sensitive at longer wavelengths [17]. With a two-photon technique, employing a femtosecond laser, the penetration depth in the biological tissue can also be increased [18]. (3) Fiber-optic light delivery is generally preferred for *in vivo* experiments, since fibers offer a high flexibility, robustness and minimize the installation and weight on the animal’s head. However, single cell resolution is difficult to achieve.

Using adaptive optics, a novel solution for technique (3) can be offered. In this article we will present a novel fiber-optic approach for single cell targeting.

1.2 Adaptive Optics System

Using adaptive optics, the DOPC in biological tissue has been studied intensively. It allows the suppression of turbidity of biological tissue to address cells at deep penetrations depths [15]. Recently, optogenetic control of a single neuron through a highly scattering skull of mice was presented [16]. Wavefront shaping by an iteratively optimized SLM was used. It was shown that sculpted light at high penetration depths can be generated. However, the temporal modulation was synchronous, meaning that only one signal can be transmitted, thus the neurons cannot be addressed individually.

Figure 1. Idea of the concept for undistorted optical transmission of two light signals through a multimode fiber (MMF). The transmission of the light signals a_1 and a_2 is accomplished by shaping the incident light at the proximal side of the MMF. In the ideal case, the spatially separated light signals $b_1 = a_1$ and $b_2 = a_2$ are received at the distal side of the MMF.

In this paper, we present an asynchronous DOPC through scattering media, which is a single multimode fiber, here. The transmission of two individual signals by a MMF using an SLM is demonstrated as depicted schematically in Fig. 1. We will investigate the following question: *Can two individually signals be transmitted through only a single fiber with high fidelity?* Our results carry potential for lensless endoscopic light delivery in biological tissue with minimal instrumental footprint. It will enable the generation of sculpted light from multimode fibers in order to activate or inhibit multiple neurons individually using optogenetics.

2. DIGITAL OPTICAL PHASE CONJUGATION

The setup used for the implementation of the asynchronous DOPC is shown in Fig. 2. The laser source is a frequency-doubled Nd:YAG solid state source (Spectra-Physics Excelsior) with a continuous-wave single-frequency power of 0.15 W @ $\lambda = 532$ nm. The DOPC is realized by a combination of a digital camera and a polarization-sensitive phase-only SLM, which constitute the sensor and the actuator, respectively. A CMOS camera (Mikrotron EoSens 4CXP, MC4082, frame rate of 280 Hz) with 2336×1728 pixel ≈ 4 Megapixel and a pixel pitch of 7 μm is used. The SLM is based on a Liquid Crystal on Silicon. It is a Pluto VIS (Holoeye Photonics, frame rate 60 Hz) and offers 1920×1080 pixel ≈ 2 Megapixel with a pixel pitch of 8 μm .

Important for a high figure of merit of the DOPC is an accurate matching of the pixels of camera and SLM. The ratio of the pixel pitch between SLM and camera is 8/7. Therefore, a magnification of 7/8 was realized by a Keplerian telescope using two lenses, allowing to eliminate the pixel size mismatch. The position and the angle-orientation of the camera and the SLM were aligned precisely in order to generate a correct phase conjugated beam.

The employed MMF (Thorlabs M14L02) of 2 m length has a step index profile, a core diameter of $D = 2R = 50 \mu\text{m}$ and a numerical aperture of $NA = 0.22$. The V-number of the fiber reads $V = \pi R NA / \lambda \approx 65$. An estimate of the number of propagating modes per polarization state is based on the equation $N_{\text{modes}} = 4V^2 / \pi^2 = 16R^2 NA^2 / \lambda^2 \approx 1710$ [19], if all modes are excited. It corresponds to the degree of freedom of the scattering media [1]. This value equals the maximum number of speckles, which is shown in the following: Considering diffraction limited focus spots with an average radius of $\lambda / (2 NA)$ at first. Due to interference effects, the minimal radius of $\lambda / (4 NA)$ results. Suppose further that the speckles with this minimal radius are uniformly distributed over the fiber core, the maximal number of speckles finally yields to $N_s = \pi R^2 / (\pi (\lambda / (4 NA))^2) = 16R^2 NA^2 / \lambda^2 = N_{\text{modes}}$. Since the speckle number corresponds to the effective pixel number, a high number of modes is required for high-resolution images.

The diagram illustrates the experimental setup for quantum communication, divided into three main sections: proximal side, MMF (Multimode Fiber), and distal side.

Proximal Side: A laser beam is directed through a half-wave plate (HWP) and a polarizing beam splitter (PBS) to a half-wave plate (HWP) and then to a beam splitter (BS1). The beam is split into two paths, labeled 1 and 2. Path 1 passes through a phase shifter $a_1(t)$ and a beam splitter (BS2). Path 2 passes through a phase shifter $a_2(t)$ and a beam splitter (BS2). The beams are then directed through beam splitters BS3, BS4, and BS5, and lenses L1 and L2, to a CMOS camera. A spatial light modulator (SLM) is also present in the proximal side.

MMF (Multimode Fiber): The optical paths from the proximal side are coupled into a multimode fiber (MMF), represented by an orange loop.

Distal Side: The optical paths from the MMF are coupled into the distal side. The beams pass through beam splitters BS6, BS7, BS8, BS9, and BS10, and lenses L3. The beams are then directed to phase shifters $b_1(t)$ and $b_2(t)$, and finally to detectors APD and CCD.

3. RESULTS

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Figure 3: Measured light intensity on the CCD at the distal side of the multimode fiber. a) The target pattern signal is projected at the calibration step. b) The output signals are received when two signals are transmitted using the multi-window digital optical phase conjugation (DOPC).

On the left, two focal spots used for the calibration corresponding to the signals b_1 and b_2 are clearly visible with a mean diameter of about $2\ \mu\text{m}$. On the right, the output of the transmission is shown, when both signals a_1 and a_2 are turned on and phase conjugation is activated. The two corresponding spots appear with a diameter of about $2\ \mu\text{m}$, too. Hence, the asynchronous multi-window DOPC is successfully accomplished. However, there is a parasitic speckle pattern as a background signal at the whole fiber facet superposed to the spots. The parasitic speckles result from the imperfection of the DOPC (especially the limited pixel number of the SLM) and is usually described using the peak-to-background ratio (PBR) [20], which is about 40 here. In Fig. 4, the measured individual spectra of both spots are displayed. It allows to estimate the crosstalk to -28 dB and -25 dB from the signals b_1 to b_2 , and vice versa, respectively.

Figure 4: Spectra of the output signals b_1 and b_2 , if the sinusoidal signals a_1 and a_2 with a frequency of f_1 and f_2 , respectively, are transmitted using multi-window DOPC. A crosstalk of -28 dB (left) and -25 dB (right) occurs due to imperfections of the DOPC.

4. SUMMARY AND OUTLOOK

We have presented a novel adaptive optical system based on a MMF and a multi-window DOPC. This technique enables a progress in light delivery for optogenetics. We have explored the transmission of two individually addressable signals through one MMF, using only a single SLM, which has not been previously reported to the best of our knowledge. The opening question of this report on the asynchronous signal transmission with high fidelity has been proven. The crosstalk of the signal transmission through a MMF depends on the figure-of-merit of the multi-window DOPC. The diameter of the generated spots is around 2 μm , which is smaller than the size of the soma of a neuron. For the digital optoelectronics advancements in the speed and the pixel number are expected [21-23]. It will foster the performance of asynchronous signal transmissions by a DOPC through scattering media. Individually addressable signals are demanded for optogenetics. A targeted illumination with both spatially and temporally controlling is of paramount importance for neural networks, since multiple individually addressable spots are needed. Using multiple signals, an individual stimulation of distinguishable cells with different temporal pattern can be accomplished. It has been shown that the presented multi-window DOPC fulfills these demands of optogenetics, enabling research to elucidate the functions of neural circuitry, but also new approaches to a treatment of brain disorder. In the future, also a contribution to understanding major neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, Huntington's disease, Amyotrophic lateral sclerosis) is possible.

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