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Adaptive Systems for Optogenetics

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Abstract

Optogenetic approaches allow the activation or the 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 Parkinson disease, epilepsy, autism and anxiety disorder as well as recovery of vision and auditory perception. A major hurdle for focusing light through biological tissue on a single neuron is the occurring scattering of the light. We demonstrate the correction of the scrambling by an optical phase conjugation using digital optoelectronic techniques.

Keywords: Optical measurement techniques, control technology, spatial light modulators, digital camera techniques, neurophotonics

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 pattern, 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.

Optogenetics

Optogenetic approaches allow the activation or the 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 unicellar alga Chlamydomonas reinhardtii to the brain of mammalians results in light-sensitive neurons [5-7]. 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, i.e., an activation of the cell. A hyperpolarization (negative charge), i.e., an inhibition/silencing of the cell can be achieved by the stimulation of Halorhodopsin (NphR) from the archea

Natronomonas pharaonis with yellow light. Then, 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 and resistance of the ion channels of neurons can be modulated by the light. It can be considered as an "optical transistor". 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.

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 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/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 [8].

Various techniques have been used to deliver light into the brain of living mammalians. (1) Implanted µLEDs 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. Thus, they are not considered further, here. (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. Adaptive optics is a solution for this problem. (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 this problem can be offered.

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 [9, 10]. Recently, optogenetic control of a single neuron through a highly scattering skull of mice was presented [11]. 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, i.e., the neurons cannot be addressed individually.

In this report, we present an asynchronous DOPC through scattering media, which is a single multimode fiber, here. The transmission of two individually 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 one fiber with high fidelity?* The hypothesis that multiple individual signals can be transmitted by employing DOPC using only a single SLM will be proven. 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.

Digital Optical Phase Conjugation

The setup used for the implementation of the asynchronous DOPC is described in this section. 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 @ λ = 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 x 1728 pixel ≈ 4 Megapixel and a pixel pitch of 7 µm is used. The SLM is a based on a Liquid Crystal on Silicon. It is a Pluto VIS (Holoeye Photonics, frame rate 60 Hz) and offers 1920 x 1080 pixel ≈ 2 Megapixel with a pixel pitch of 8 µm. Important for the 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 has been 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.

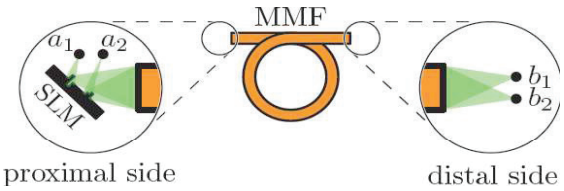

Fig. 1: Idea of the concept for undistorted optical transmission of two light signals though a multimode fiber (MMF) using a spatial light modulator (SLM). The transmission of the light signals a_1 and a_2 is accomplished by shaping the incident light at the proximal side of the MMF. In the ideal case,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

The employed MMF (Thorlabs M14L02) of 2 m length has a step index profile, a core diameter of D = 2 R = 50 µm and a numerical aperture of NA = 0.22. The V-number of the fiber reads $V = \pi\ 2\ R\ NA\ /\ \lambda \approx 65$. An estimate of the number of propagating modes per polarization state is based on the equation $N_{modes} = 4\ V^2\ /\ \pi^2 = 16\ R^2\ NA^2\ /\ \lambda^2 \approx 1710$ [12], if all modes are excited. It corresponds to the degree of freedom of the scattering media [13]. This value equals the maximum number of speckles, which is shown in the following: Considering diffraction limited focus spots with an average radius of λ / (2 NA) at first. Due to interference effects, the minimal radius of λ / (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 $N_S = \pi\ R^2\ /\ (\pi\ (\lambda\ /\ (4\ NA))^2) = 16\ R^2\ NA^2\ /\ \lambda^2 = N_{modes}$. Since the speckle number corresponds to the effective pixel number, a high number of modes is required for high-resolution images.
The complex phasor of the beacons is captured by off-axis holography at the CMOS camera with a single acquisition. The sought first diffraction order is separated from the zeroth diffraction order and the twin minus first diffraction order in the spatial frequency domain (angular spectrum method). An angle of approximately 2° was adjusted between the reference beam and the scattered beam from the MMF. The calculated phase of the off-axis hologram is inverted by a PC and projected to the SLM. The reflected phase conjugated light propagates backwards through the MMF and generates two foci at the distal end. The DOPC was realized at two windows, i.e. separate areas of both the camera and the SLM.

Results

The laser light phase for both individual input signals a_1 and a_2 is modulated employing the SLM at the proximal side using the inverse of the phase measured by digital holography. The signals are generated using two optical choppers with a fundamental signal frequency of $f_1 = 220$ Hz and $f_2 = 170$ Hz. The output signals b_1 and b_2 at the distal side of the fiber are measured using avalanche photodiodes (APD). In addition, the resulting light intensity at the distal side of the MMF is measured at the CCD, see Fig. 2.

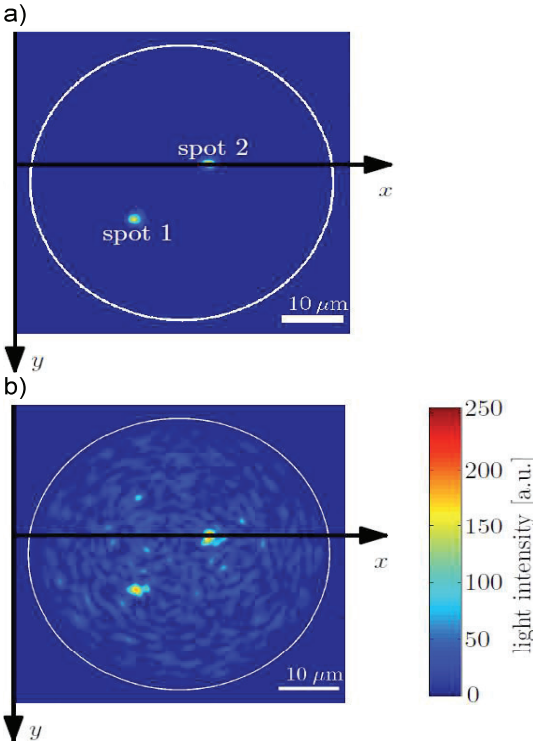

Fig. 2: Measured light intensity on the CCD at the distal side of the multimode fiber. a) The target pattern signal is projected at the calibration step. b) The output signals are received when two signals are transmitted using the multi-window digital optical phase conjugation (DOPC).

On the top, 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 µm. On the bottom, the output of the transmission is given, when both signals a_1 and a_2 are turned on. The two corresponding spots appear with a diameter of about 2 µm, 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 which also overlaps the spots. The parasitic speckle results 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) [14]. The PBR is about 40 and results in a crosstalk of -28 dB and -25 dB from the signals b_1 to b_2, and vice versa, respectively, which is indicated in Fig. 3.

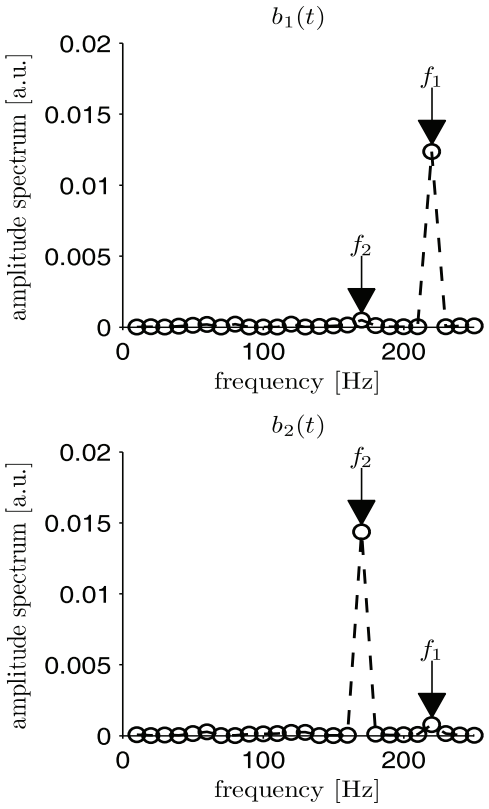

Fig. 3: Spectra of the output signals b_1 and b_2, if the sinusoidal signals a_1 and a_2 with a frequency of f_1 and f_2, respectively, are transmitted using multi-window DOPC. A crosstalk of -28 dB (left) and -25 dB (right) occurs due to imperfections of the DOPC.

Summary

We have presented a novel adaptive optics system based on a MMF and 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 one 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 answered with experimental and theoretical studies. The crosstalk of the signal transmission through a MMF depends on the figure-of-merit of the multi-window DOPC. For the digital optoelectronics advancements in the speed and the pixel number are expected. 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 can fulfill these demands of optogenetics.

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