Holographic photo-stimulation for dynamic control of neuronal population activity

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***Abstract*— Spatiotemporal patterns of activity carried across large populations of neurons are the fundamental representation of information within the nervous system. Patterned optical photo-stimulation of neural populations provides a general strategy for controlling such spatial-temporal patterns, but previous realizations of this technology did not allow high-rate, parallel, light-efficient control of large neural populations. To address this challenge, we have been developing a new generation of holographic pattern photo-stimulation systems that use phase-only spatial light modulators (SLM) to create computer-controlled light patterns in two and three dimensions. SLMs use phase modulation and light diffraction to provide a light-efficient method for flexibly creating desired light patterns which can be switched in our systems in millisecond timescales. Holographic photo-stimulation provides a powerful strategy for dynamic patterned photo-stimulation of neural populations and could be used in research and neuroprosthetic interfaces.**

Keywords-holography; SLM; two-photon; ChRII; optical control; high-rate photo-stimulation.

I. INTRODUCTION

The advent of optical methods for neural stimulation has gained much momentum over the last few years. Optical-based neural interfaces generally have higher spatial resolutions when compared with electrode-based interfaces. Their use also solves the problem of mechanical tissue damage which is inherent to electrode usage.

Neuronal excitation by light was first demonstrated in the 1960's [1,2]. Several new techniques have been devised and used in recent years. For example, caged compounds, which are inactive in their original conformation, undergo uncaging when they absorb light (typically in the UV region), thus driving neural activity. Another class is the optogenetic methods, which employ genetically engineered photo-activatable probes, such as Channelrhodopsin II (ChR2). When these are introduced into neurons, they are able to target a specific cell group with high selectivity. Upon illumination with light of a wavelength matching the probes' absorption spectrum, neural activity can be elicited. In a third method introduced by Wells *et al.* [3,4], a low-power, pulsed IR laser was used to induce neural stimulation without causing tissue-damage.

To imitate physiology, a good photo-stimulation method must be capable of stimulating multiple neurons simultaneously and allow for a change of stimulation patterns at a sufficiently high frame rate. The first generation of systems for patterned photo-excitation of neurons was based on deflecting a laser beam in a random-access fashion. Our earlier acousto-optical uncaging system could address 20,000 locations per second (see Fig. 1(a)) [5]. These methods, however, offer little in terms of simultaneous photo-stimulation of multiple areas. Moreover, during the majority of the scanning period, the beam "travels" *between* the points of interest, leaving little time for the stimulation itself.

By contrast, the second generation of systems allowed for parallel photo-stimulation by using digital projector technology such as digital micromirror devices (DMDs) (Fig. 1(b)). The principle behind these systems is an amplitude-only modulation of the input beam; that is, by blocking certain parts of the beam, it is possible to generate the desired illumination formation in the focal plane. Unfortunately, this process entails significant power loss.

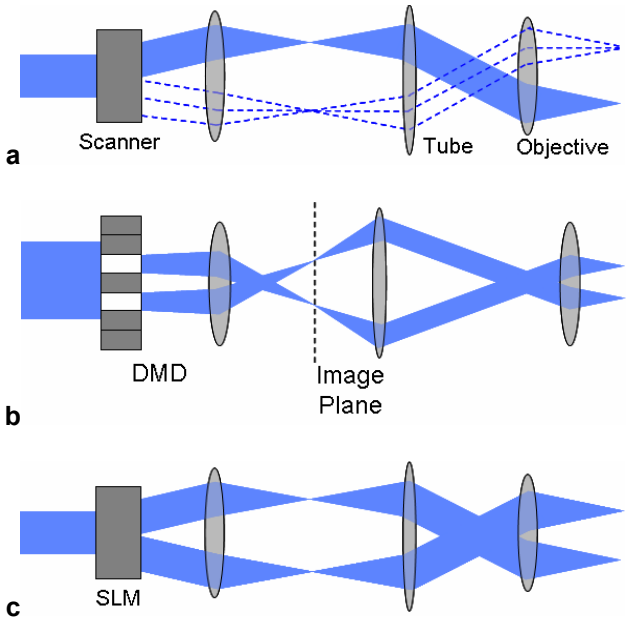

Figure 1. **Principle operation methods of different generations of photo-stimulation systems.** (**a**) Acousto-optic systems, (**b**) a Digital Micromirror Device system, (**c**) a Spatial Light Modulator system.

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We present here the third generation of systems, based on Spatial Light Modulators (SLMs) (Fig. 1(c)). An SLM is a liquid-crystal device which acts as a wavefront modulator. Essentially, it is a pixelized retardation plate, with each pixel imposing a user-controlled retardation. By modulating only the phase of the incoming laser beam, leaving its amplitude almost intact, SLMs can produce desired holographic stimulation patterns with the advantage of having no power lost due to partial beam blocking, as is the case with amplitude-only modulation. By using an SLM in such a phase-only configuration, it is possible to target specific areas or cells of interest for photo-stimulation without scanning. Additionally, the phase-image addressed to the SLM can be typically refreshed at a frame rate high enough to enable dynamic control of a population.

II. Theory: Principles of SLM Operation

The mathematical relation between the electromagnetic field at the plane of the SLM (back focal plane), $U_{SLM}(u,v)$, and at the image plane (front focal plane), $U_{image}(x,y)$, is essentially that of a Fourier transform [6] (see Fig. 2):

$$U_{image}(x,y) = \frac{1}{\lambda f}\iint U_{SLM}(u,v)\cdot e^{-j\frac{2\pi}{\lambda f}(u\cdot x+v\cdot y)}dudv$$
$$= \frac{1}{\lambda f}\mathbb{F}\left[U_{SLM}(u,v)\right]\left(\frac{x}{\lambda f},\frac{y}{\lambda f}\right)$$

Thus, assuming $U_{image}(x,y)$ is the pattern of interest, it suffices to calculate its inverse Fourier transform in order to get the phase-image needed on the SLM. Apparently, however, a problem arises when working in the desired phase-only modulation mode, as this mode leaves the amplitude of the field in the SLM plane intact, with the pixelized retardation plate affecting only the phase of the input beam. This is equivalent to discarding the original amplitude in the inverse Fourier (SLM) plane and attaching to the remaining phase an amplitude profile which matches that of the input beam (approximately Gaussian).

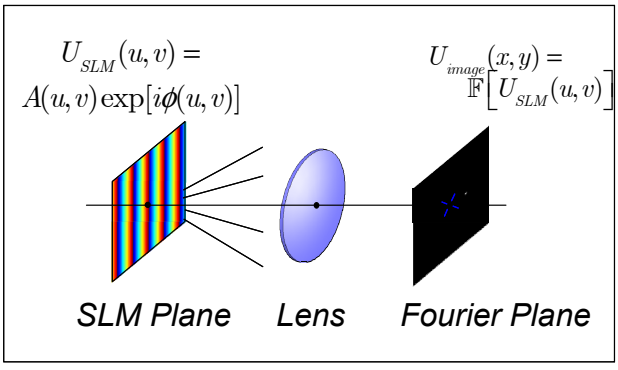

Figure 2. **The relation between an image at the front focal plane (image plane) and the back focal plane (SLM) is that of a Fourier transform.**

However, experimentally, it has been shown that the amplitude values are of lesser importance than phase values, rendering the loss of amplitude almost insignificant in terms of the resulting pattern. To demonstrate this intuitively, think of a digital image, $I[m,n]$, as a weighted sum of displaced Kronecker's deltas:

$$I[m,n] = \sum_q\sum_p \alpha_{p,q}\delta[m-p,n-q]$$

Fourier transforming it yields:

$$\mathbb{F}\left\{\sum_p\sum_q \alpha_{p,q}\delta[m-p,n-q]\right\}[k,l] =$$
$$= \sum_p\sum_q \alpha_{p,q}e^{-i2\pi\frac{(kp+lq)}{NM}}$$

The above result shows that the main information regarding the 'whereabouts' of each Kronecker's delta is encoded in the phase of the Fourier plane, which makes the Fourier phase more significant for image reconstruction purposes. Figure 3 shows some phase-image and real-image pairs simulations.

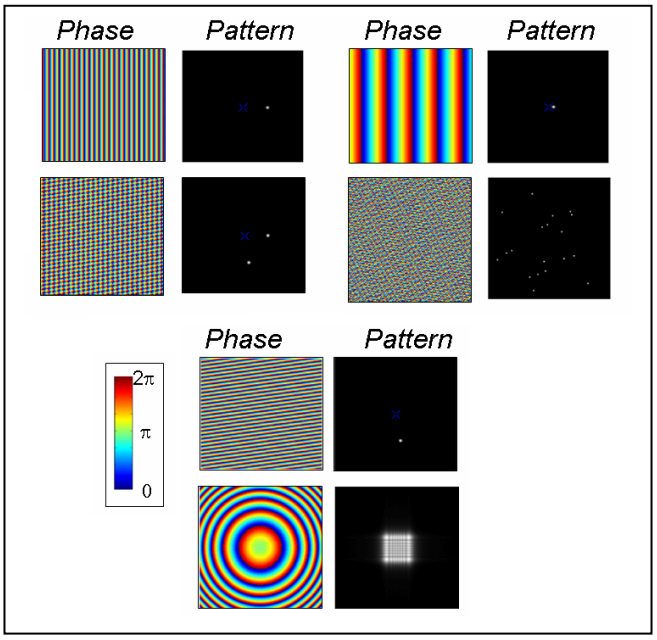

Figure 3. **SLM phase images and their corresponding theoretical stimulation patterns in the focal plane.**

In practice, however, a mere inverse Fourier transform is usually insufficient. In addition to the distortion caused by dumping the amplitude values, other significant problems arise in the forms of high-order diffractions and ghost images – undesirable artifacts which are reflections of the original desired pattern. The ghost problem is further exacerbated by the fact that, being computer-controlled, the SLM can only introduce values from a quantized set of phase retardations at each pixel. Therefore, if more accurate holograms are sought, it is possible to employ one of several iterative algorithms designed to tweak the phase-image in order to generate a better image and redistribute the input light so that more of it is directed to the first-order pattern. Examples for such algorithms

are the Gerchberg-Saxton algorithm, the improved GSW algorithm proposed by Di Leonardo et al. [7] or the Generalized Adaptive Additive algorithm.

Another major noise sources in holographic illumination is the formation of speckles. Speckle-like variations cause the illuminated pattern to be nonuniform in appearance. Golan and Shoham [8] were recently able to overcome this problem by means of shifting and averaging (see Fig. 4).

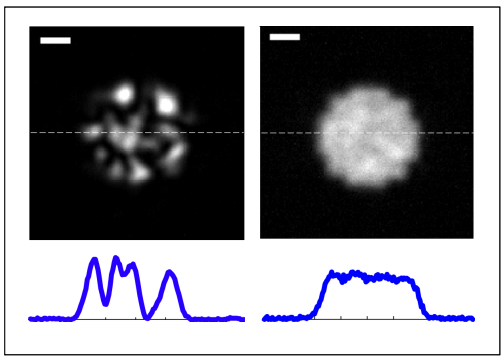

Figure 4. **Speckle elimination by shift-averaging** (adapted from Golan & Shoham, Optics Express). Image of a spot before speckle elimination (*left*), and after speckle elimination (*right*).

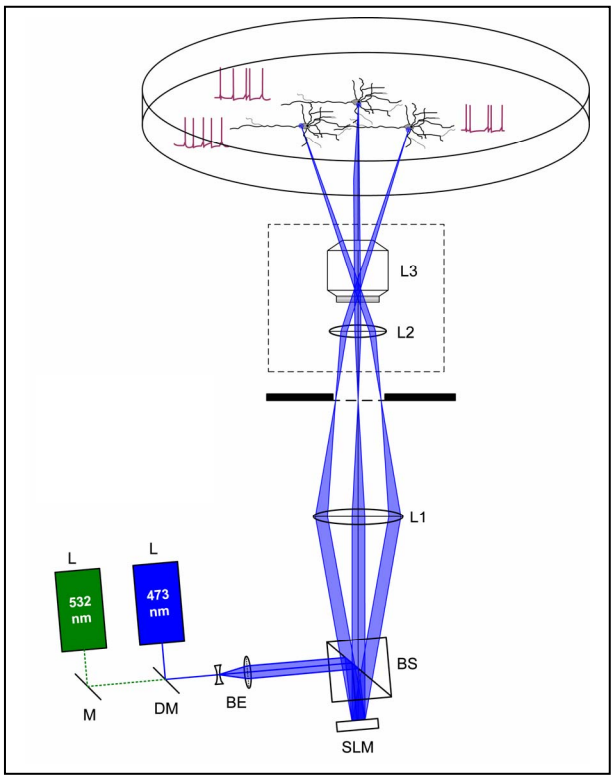

Figure 5. **Schematic drawing of the one-photon optical setup**, which includes two laser sources emitting two different central wavelengths. The laser beam is first expanded via a beam expander, then diverted toward the SLM using a polarizing beamsplitter. The emerging beam is imaged onto the back focal plane of the objective lens, whereby it is focused at the plane of interest for stimulation.

III. Methods and Results

A. *One-photon system*

In this system, a ForthDD ferroelectric SLM with a refresh rate of over 1kHz with two distinct phase levels was incorporated into an inverted microscope system. Two laser sources (DPSS @ 473 nm and @ 532 nm) were used for illumination. Figure 5 shows a schematic drawing of the system.

This system also has a relatively high diffraction efficiency. This, coupled with parallel stimulation and the fast refresh rate, yields a system with the theoretical capacity of stimulating about 1 million spikes per second, using only a few milliwatts of power, since each spike requires only a few nano-Joules of optical energy.

It should be noted that the size of the light spot at the focal plane is a critical parameter of the stimulation system. For individual cells stimulation, it is desirable to match or overshoot the size of a cell's soma. The spot size may be changed either by varying the beam diameter or the axial displacement, or by tiling adjacent spots.

Another possible use of such a double-laser system is time-sharing of different wavelengths for simultaneous (or quasi-simultaneous) activation of different light-sensitive ion channels, which should enable a more realistic control of the neural activity.

B. *Moving to 3D: Two-photon system*

Multi-photon techniques have the advantage of inherent optical sectioning over single-photon techniques. In one-photon illumination, the illuminated volume extends considerably above and below the focus plane of interest, yielding undesired photo-activation or fluorescence in these areas and compromising axial resolution. In two-photon, the illumination volume is largely confined to the focus plane.

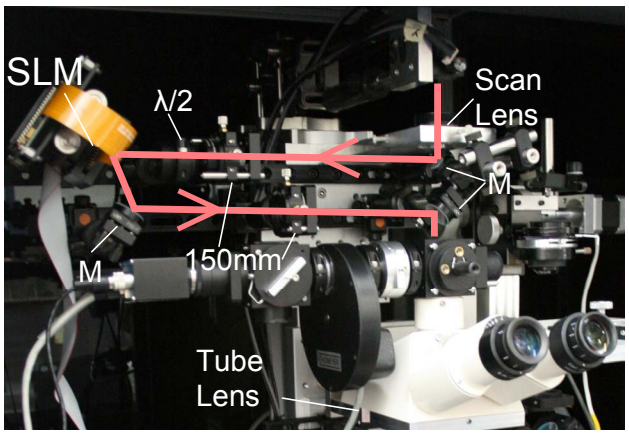

Figure 6. **Image of two-photon system.** As in the one-photon system, the beam is first expanded. It is then reflected off the SLM after a half-waveplate matches the polarization to the parallel nematic SLM. The SLM plane is then imaged onto the objective's back aperture (not shown), and then focused to the image plane.

By mathematically adding Fresnel lens terms to the computed phase-image, it is possible to shift the pattern, or parts of it, in both directions relative to the focal plane of the objective lens [9]. Thus, because of the aforementioned inherent sectioning, one can design three-dimensional patterns of interest around the focal plane, thereby enhancing the pattern in terms of semblance to the areas of interest in the sample.

In our two-photon system, we incorporated a BNS XY Phase Series parallel nematic SLM with a maximal refresh rate of about 100 Hz. While this SLM has a considerably lower refresh rate, it allows for 8-bit quantized phase values in each pixel of the SLM, which should enable the creation of more accurate holographic patterns in the output, and partially alleviate the ghost problem. **Figure 6** shows an image of this system.

To test our system in terms of capability of producing good patterns, we took a fluorescent image of a cell culture, and produced several holographic patterns resembling different cells in the image. The results, shown in red in Figure 7, show quite a good match between the real image and the holographic patterns.

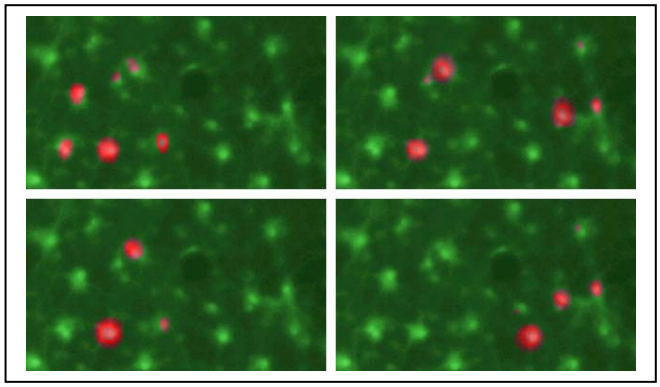

Figure 7. **Comparison of the stimulation patterns to the actual image of the neural network.** In each frame, a different group of cells is targeted.

IV. Discussion

Spatial Light Modulators are a promising tool in neural engineering, particularly for simultaneous photo-stimulation in dynamic multi-spot patterns. This should facilitate the control of neural population activity.

In recent independent works by two other groups, photo-stimulation systems based on SLMs were also developed. Lutz *et al.* [10] demonstrated one-photon holographic photolysis of caged glutamate in brain slices and Nikolenko *et al.* [11] have implemented an SLM-based photo-stimulation system using two-photon uncaging of caged glutamate. Papagiakoumou *et al.* [12] have very recently incorporated an SLM into a system capable of spatiotemporal shaping of ultrashort laser pulses.

Our one-photon system is theoretically capable of producing about one million spikes in one second using only a few milliwatts, and can change the produced patterns at very high rates. It also allows for time-sharing between different excitation wavelengths. On the other hand, our two-photon system is capable of producing patterns closely resembling neural soma structures, and can produce three-dimensional holograms for better excitation. Both our systems are compatible with any of the optical excitation methods described in the Introduction section.

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