GPC-modalities for Neurophotonics and Optogenetics

Glückstad, Jesper; Eriksen, René L.; Madsen, Andreas Gejl

Published in:
Complex Light and Optical Forces XV

DOI:
10.1117/12.2583507

Publication date:
2021

Document version:
Final published version

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark.
Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim.
Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 14. Sep. 2023
GPC-modalities for neurophotonics and optogenetics

Glückstad, Jesper, Eriksen, René, Madsen, Andreas Gejl

GPC-modalities for Neurophotonics and Optogenetics

Jesper Glückstad1,2, René L. Eriksen1 and Andreas G. Madsen1
1SDU Centre for Photonics Engineering, Mads Clausen Institute, Univ. Southern Denmark
2OptoRobotix ApS, DK-2000 Frederiksberg, Denmark

email: jegl@mci.sdu.dk

ABSTRACT

The powerful discipline of optogenetics can be described as “the branch of biotechnology which combines genetic engineering with optics to observe and control the function of genetically targeted groups of cells with light”. Using advanced laser beam-shaping such as Generalized Phase Contrast (GPC) and derived holographic modalities makes it possible to take advantage of cutting-edge two-photon technology to develop an unprecedented ‘circuit optogenetics’ platform with both high spatio-temporal selectivity and high penetration depth without disturbing speckle-noise. We will report on the latest laser shaping GPC-modalities with temporal focusing to uniquely sculpt patterns of one- and two-photon excitations for living neural-circuits and genetically modified light-sensitive cells.

Keywords: Generalized Phase Contrast (GPC), Neurophotonics, Optogenetics

1. INTRODUCTION

The emerging biophotonics discipline of optogenetics can be described as “the branch of biotechnology which combines genetic engineering with optics to observe and control the function of genetically targeted groups of cells with light”. Using advanced laser beam-shaping such as Generalized Phase Contrast (GPC) makes it possible to take advantage of cutting-edge two-photon technology to develop an unprecedented ‘circuit optogenetics’ platform with both high spatio-temporal selectivity and high penetration depth without disturbing speckle-noise in e.g., living neural tissue. Light sculpting GPC-modalities with temporal focusing can be used to uniquely sculpt patterns of both one- and two-photon excitations for living neural-circuits and genetically modified light-sensitive cells. Recently, we are extending our research into developments of new Holographic GPC-modalities to match ultrafast-pulsed light sources in temporal focusing geometries.

2. GENERALIZED PHASE CONTRAST (GPC)

The Generalized Phase Contrast method (GPC) belongs to a class of non-absorbing common path interferometers [1]. A phase transmittance distribution directly representing the desired output intensity is imaged through the interference with a reference wave synthesized from phase-shifted low spatial frequencies. This is implemented using a phase contrast filter (PCF) whose phase shifting region is situated at the center of the optically Fourier transformed input. Static GPC light shapers have been implemented with binary phase plates and have been used for efficiently illuminating spatial light modulators (SLM) [2]. Likewise, dynamic GPC is implemented using a programmable phase-only SLM and has been used in experiments such as optical trapping, two-photon excitation and neuro photonics [3].

Unlike diffractive approaches, GPC uses the target shape as the phase mask that directly interfaces with the incident Gaussian, instead of a pattern based on the target’s Fourier transform (e.g. holograms). This makes GPC robust to input beam variations as convolution or other spatial filtering effects are avoided. The use of a common path configuration renders steep well-defined edges in the shaped output. Furthermore, the target
output shapes could easily be replaced through interchangeable phase masks or by simply redrawing the pattern encoded into the SLM. GPC’s use of an imaging geometry makes it advantageous with techniques that use multiple wavelengths, pulsed or broadband sources. One example is temporal focusing which effectively confines light along the axial direction and is therefore relevant in printing 2D slices in Laser Direct Writing or for exciting neurons in two-photon based optogenetics experiments [3]. Temporal focusing temporarily stretches a pulsed light source such that the intensity is lowered except at the focal plane. This means for creating isolated speckle-free “light sheets” have been used for two-photon excitation of selected neurons at an isolated plane within the tissue volume. If the biological tissue were instead replaced by photo-resist, GPC combined with temporal focusing would be able to solidify a 2D slice without affecting the planes adjacent to that slice.

Figure 1. A GPC-system can efficiently transform an incident Gaussian beam into a bright rectangle. Besides a standard telescopic $4f$ setup formed by the two Fourier lenses, GPC uses a simple binary phase mask at the input and phase contrast filter at the Fourier plane. An amplitude masking configuration is shown besides to illustrate the significant difference in energy utilization when producing the same shaped output (Adapted from ref. [4]).
We have previously shown theoretically [5] and experimentally [6] that GPC shows robustness to shift in wavelength and can maintain both projection length scale and high efficiency over a range 0.75\(\lambda_0\) - 1.5\(\lambda_0\) with \(\lambda_0\) as the characteristic design wavelength. With the resulting performance across multiple wavelengths and the recent availability of tabletop super-continuum lasers, GPC light shaping opens the possibility for creatively incorporating various multi-wavelength approaches into spatially shaped excitations that can enable new broad-band light applications.
Figure 4. GPC-projections using a supercontinuum laser displayed by color CCD-imaging. The power at different wavelengths are adjusted individually for visibility. The input Gaussian beam size exhibits some wavelength dependence. Adapted from ref. [6].

3. HOLOGRAPHIC GPC

We have previously shown how to modify a GPC-setup to produce output spots instead of the mapped phase images by using matched filtering [7, 8]. In addition to the phase shifting circular region at the PCF, concentric phase shifting rings are applied to the Fourier distribution to match the alternating lobes of the Airy disk that correspond to specified input circles at the input phase mask. This modified GPC embodiment, consisting of a matched filter at the Fourier plane, is thus called matched filtering GPC (mGPC). While being relatively simple to implement, mGPC does not fully exploit the benefits of digital holography. Both GPC and mGPC, only have a fixed element at the Fourier plane which is the PCF or a matched filter. This fixed Fourier filter makes both of them practical to implement requiring only a single SLM to dynamically reconfigure the output. The fixed matched filter in mGPC also limits the features that it can borrow from 2f digital holography. In a typical 2f holographic setup, a dynamic SLM is behind the Fourier transforming lens. In GPC or mGPC, the 2f part defined by the region between the PCF or matched filter and the output beams, on the other hand, has a “2f input” that is not as dynamic as an illuminated SLM.

As suggested by general Fourier optics considerations, there are far more possibilities if the phase element at the Fourier plane can also be dynamically controlled. Indeed, near ideal arbitrary complex fields can be generated when using a tandem of two dynamic SLM surfaces. Hence, recently we have developed Holographic GPC that takes advantage of some features of a so-called “tandem configuration” by having a phase modulator at the spatial frequency or Fourier plane. However, similar to GPC and mGPC, Holographic GPC maintains its practicality of requiring just one active SLM. Thus, to maintain this practicality and economy, the first phase modulating element is a well defined, easily fabricated, and generally reusable phase mask instead of another SLM as the tandem configuration suggests. In the usual tandem configuration, the first phase element being a hologram generally bears no resemblance to the final output and would require recalculation for different outputs. Pre-fabricated phase elements would be impractical as they cannot be specified with a few parameters, require multiple phase levels to be efficient and would have to be replaced for each new output configuration.

Just as the shape of the generated individual output beams can be important, so is the shape of the light that is illuminating a hologram. Ultimately, the read-out illumination determines the amplitude and phase distribution of the point-spread function (PSF) at the output optical far-field plane. We are particularly interested in modifying the “spread function” of the output beams. As the typical illumination shape would have a Tophat or a Gaussian distribution, the typical PSFs are either Airy-disk or Gaussian shaped. The target output PSF can
thus be changed by illuminating with an (inverse) far-field transformed beam shape. The challenge, therefore, is the efficient creation of an initial basis beam shape (typically using the Fourier transform) that will become the output’s PSF.

It is well known that a Fourier transformed amplitude mask can be used to illuminate a hologram in order to get PSFs with the same amplitude pattern. Holographic GPC starts with a similarly looking phase mask that efficiently transmits the input light without absorbing photons. But unlike a straightforward convolution, phase filtering is required to convert the phase mask into shaped PSFs at the output. As it relies on GPC’s phase to intensity mapping, Holographic GPC also inherits GPC’s efficiency advantage over amplitude masking and would in principle also have 3x brighter PSFs or over 90% of energy savings.

To understand how Holographic GPC works, we first briefly revisit the standard GPC configuration and subsequently identify the modifications necessary to make multiple holographic copies of this GPC output. In a standard GPC setup (i.e. Figure 5), the input phase mask is first optically Fourier transformed and the resulting distribution is focused on a phase contrast filter (PCF). An output intensity mapping of the input phase mask is generated from the interference of the imaged input with a so-called synthesized reference wave (SRW) that results from the low frequency components phase shifted by the PCF.

We treat the region between the PCF plane, through the imaging lens, then to the output intensity (Fig. 5(d) to (g)), as a Fourier transforming configuration. The optical Fourier transform of the input phase mask can be thought of as the illumination at this plane. For a rectangular or circular input phase mask, this PCF illumination typically resembles a Sinc-function, or an Airy disk, but in general, this can be the Fourier transform of the desired PSF-shape with similar geometry as the input phase mask. As with holography, the PCF’s phase shift is correcting the central part, such that it matches the phase distribution of an ideal Sinc function, Airy disk or Fourier transform of the desired PSF pattern. Hence, through convolution, by placing a hologram phase on top of this PCF-shifted, Fourier-transformed phase mask, Holographic GPC can produce a beam distribution wherein each beam takes the form of the intensity mapped input illuminated phase mask as in Figure 6.

As Holographic GPC operates by efficiently modifying the point spread function, the individual beams are identical copies of the intensity-imaged phase mask pattern. This multi-beam approach is a different paradigm from standard GPC wherein a phase mask utilizes multiple smaller sub-shapes that need not be identical, and hence the corresponding output individual beams can also have different shapes. However, unlike standard GPC or mGPC, Holographic GPC’s output beams are not constrained to an imaging plane, but rather, can be addressed in a 3D holographic manner. Furthermore, a compensating phase mask region is not necessary for maintaining the optimal fill factor while changing the number of output beams.
Figure 6. Holographic GPC setup. Compared to standard GPC, a hologram (e) is placed in addition to the PCF at the Fourier plane of the first lens. For practical implementations, the hologram is typically encoded on an SLM, and the sizes of the input beam, phase mask, PCF and focal lengths have to be adjusted. The second lens (g) optically Fourier transforms the light that is altered by the hologram and PCF to get a distributed output consisting of speckle-free contiguous shapes. Adapted from ref. [10].

4. NEUROPHOTONICS CONSIDERATIONS

Today optogenetics is predominantly performed with visible light sources. However, it has been shown [3] that the use of two-photon processes induced by near-infrared light pulses enables penetration much deeper into a tissue structure with a high degree of spatial selectivity.

Figure 7. Femtosecond laser GPC-generated patterns with temporal focusing encoded for parallel two-photon optogenetics excitation. Adapted from refs. [3,9,11].
Stimulation with visible light does not put strong requirements on the light sources, but faces challenges in delivery and thereby selectivity, limiting the practical use in a variety of biological systems. Visible light is strongly scattered by tissue and cellular structures, so unless the light is delivered in close proximity to the targeted cells, one loses both spatial selectivity and signal intensity. Typical solutions for e.g., imaging in human tissue samples involve using very thin tissue slices or the introduction of optical fibers directly into the tissue of interest. The photoconductive opsins can also be stimulated by near-infrared light through two-photon processes. Light scattering and absorption in tissue is much lower at near-infrared wavelengths giving this technique a great advantage in penetration depth and selectivity. Using advanced beam-shaping techniques makes it possible to take advantage of the two-photon technology and develop an unprecedented optogenetics technology with both high spatiotemporal selectivity and high penetration depth. Our future aim is to apply newly developed Holographic GPC modalities that make it possible to take advantage of cutting-edge two-photon technology to develop an unprecedented ‘circuit optogenetics’ platform with both high spatio-temporal selectivity and high penetration depth without disturbing speckle-noise. A key aim will be for the first time to laser-address a circuit consisting of a triple-digit number of neurons simultaneously.

REFERENCES