Two-photon optogenetics by wave front shaping of ultra fast pulses
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Neurophotonicstics:

Development and use of advanced optical methods for Neurobiology

Imaging and functional imaging

- Patterned endomicroscopy
- Confocal
- STED

Optical stimulation

- Photolysis
- Optogenetics

Sub cellular (~30 nm)

- STED microscope

In vivo

- Micro-endoscope

A fundamental task in neuroscience research is to establish a map of the neural connections within the brain.

**Electrode stimulation**

Some experimental challenges:
- mechanical damages
- limited spatial resolution
- difficulty in inhibiting neurons

**Light stimulation**

- not invasive
- spatial and temporal resolution
- flexible
- reversible
An alternative way to stimulate neurons is to use light.

The use of light is not invasive permits to precisely control the location, the time and the strength of stimulation, is flexible and reversible.

Richard Fork

Laser Stimulation of Nerve Cells in Aplysia

Abstract. Laser radiation at 488 nanometers selectively stimulates neurons in the abdominal ganglion of the marine mollusk Aplysia californica. The laser radiation can be scanned over the surface of the ganglion and can be effectively utilized in mapping cellular interconnections. The laser appears to cause these changes through some mechanism other than damage.

(R. Fork, Science, 1971)
Optogenetics: Light gated channels and pumps

The demonstration of functional expression of Channelrhodopsin-2 in mammalian (Nagel et al. 2003) and neuronal cells (Boyden et al. 2005) marked the beginning of optogenetics

Channelrhodopsin ChR2: excitation

Halorhodopsin NpHR: inhibition

Chlamydomonas reinhardtii (algae)

Natronomonas pharaonis (archaeobacteria)

Zhang et al. (2006)

Zhang et al. (2007)
Examples: excitation

- Blue light stimulation of the right secondary motor cortex in transgenic mice expressing ChR2 (Thy1::ChR2-EYFP)
Examples: inhibition

- Transgenic C. elegans expressing NpHR in muscles

INTRODUCTION:

Key biological questions have been already addressed with simple illumination methods based on wide-field blue light illumination.

To individuate subsets of genetically identical connected cells, or establish the role of specific spatiotemporal excitatory patterns in guiding animal behavior, more sophisticated illumination methods are required.
Two-photon excitation

Improvement in axial resolution because of non-linearity of the two photon effect

Improvement in penetration depth because of the use of longer wavelength

\[ S_{2PE} \propto \frac{I^2}{\tau \cdot f} \]

B. Amos Cambridge
Two-photon photoactivation

conventional two-photon excitation volume is too small:

• Laser scanning

Light gated channels (ChR2)

• Low conductivity (∼80 fS)
  (Feldbauer et al PNAS, 2009)
• Low density of channels

→ Not enough channels to generate an AP
First demonstration of 2P ChR2 activation in cultured neurons

- First demonstration for AP generation in cultured neurons
- High two-photon cross section (~250 GM at 920 nm)

(coords)

Limited temporal (~30ms for single cell excitation) and axial resolution
conventional two-photon excitation volume is too small:

- **Parallel approach**

Light gated channels (ChR2)

- Low conductivity (~80 fS)  
  \((\text{Feldbauer et al PNAS, 2009})\)
- Low density of channels  
Parallel approach: Gaussian beam

- Poor Axial resolution
- Limited to circular shape

Ideally:
- efficiency
- multi-scale excitation
- ms temporal resolution
- µm axial resolution

axial resolution = \( \frac{K \cdot \lambda}{NA^2} \propto \frac{s^2}{\lambda} \)
Optical techniques:

- Digital holography
- Generalized phase contrast method
- Temporal focusing

Results:

- 2P optogenetics in brain slices
- Propagation of shaped beams through scattering media
An efficient way of controlling light distribution is to modify its optical wave front by pure phase modulation (not losses of power).

**INTRODUCTION: phase modulation**

Liquid crystal based modulators (SLM)

![Diagram of liquid crystal molecules with electric fields and phase changes](image)

- Multi spot generation
- Spot shaping
**INTRODUCTION: phase modulation**

Phase-only modulation is obtained by modifying the thickness of the diffractive elements.
Digital holography: Gerchberg and Saxton algorithm

Objective back aperture

Liquid crystal based modulator

Focal plane

$U(x_1, y_1) = A(x_1, y_1) e^{i \Phi(x_1, y_1)}$

$U(x_0, y_0) = A(x_0, y_0) e^{i \Phi(x_0, y_0)}$

$U(x_0, y_0) = F(U(x_1, y_1))$

$U(x_1, y_1) = F^{-1}(U(x_0, y_0))$

Gerchberg and Saxton algorithm [Optik (1972)]
Digital holography: Gerchberg and Saxton algorithm [Optik (1972)]

1. Building starting function
   - Random phase
     \[ \Phi(x_0, y_0) \]
   - Target illumination
     \[ |U(x_0, y_0)|^2 = \]
   - \[ g(x_0, y_0) = |U(x_0, y_0)| e^{i\Phi(x_0, y_0)} \]

2. Inverse Fourier transform
   \[ G(x_1, y_1) = \text{FT}^{-1} g(x_0, y_0) \]

3. Retain phase, Replace amplitude
   \[ \Phi(x_1, y_1) \]
   \[ |G(x_1, y_1)| = |U(x_1, y_1)| \]

4a. Retain phase, Replace amplitude with target
   \[ g_1(x_0, y_0) = |U(x_0, y_0)| e^{i\Phi'(x_1, y_1)} \]

4b. STOP

\[ \Phi(x_1, y_1) \]

Evaluation step
\[ E'^2 = \sum_{x, y} [g'_1(x_0, y_0) - |U(x_0, y_0)|]^2 \]

Output phase
\[ \Phi(x_1, y_1) \]


Digital holography: Gerchberg and Saxton algorithm [Optik (1972)]

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RESULTS:


E. Papagiakoumou, et al., Optics Exp. (2008)
E. Papagiakoumou, et al., Optics Exp. (2009)

F. Anselmi et al. PNAS (2011)
Digital holography: The set up

Side view

Front view

Objective conjugate plane

Wave front analyzer

Beam expander

SLM

Laser (405 nm)
**Summary: Digital holography**

Digital holography

- Phase modulation: minimize intensity losses
- Parallel approach: temporal resolution fixed by the dwell time
- Intrinsic optical sectioning: $s \propto \Delta z$
- Possibility for 3D patterning
The generalized phase contrast method (GPC)

Extension of phase contrast method (Frederik Zernike 1930, Nobel prize 1953)

*Phase map transformed into an intensity map*
Expansion of the phase contrast method (Zernike 1932) to $[0, 2\pi]$ phase variation:

$$\exp(i\phi(x, y)) = \left(\iint dx\,dy\right)^{-1} \iint \exp(i\phi(x, y))\,dx\,dy$$


$$\theta = \pi \quad I \approx 2|1 - \cos\phi(x, y)|^2 \quad [0; \pi] \rightarrow [0; 4]$$
Two photon-GPC: Results

$\lambda_{\text{exc}} = 780 \text{ nm}$

Fluorescence

Phase mask

Laser pattern

DH

SLM plane = Fourier plane

Focal plane

GPC

SLM plane = conjugate plane

Focal plane
**Diffraction efficiency and excitation field: DH and GPC**

- DH, diffraction efficiency limited by SLM pixel size: \((\sin X/X)^4\)
- GPC, given by the interferometer characteristics:

\[
F = \frac{A_{\pi}}{A_{\pi} + A_0}
\]

---

Practical challenges to set up GPC+TF:

- Alignment of phase dot (Wave front analyzer)
**Practical challenges to set up GPC+TF:**

- **SLM=FOV:** \( A_{\text{spot}} \sim \frac{1}{4} \text{FOV} \)

- **Iris:** \( F = \frac{A_{\pi}}{(A_{\pi} + A_0)} = \text{constant} \)

- **Tilt to compensate for grating tilt**

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*E. Papagiakoumou et al. Nature Methods (2010)*
**Axial propagation?**

**Gaussian beam**
- Back aperture
- Axial resolution, $b \propto s^2$

**GPC**
- Back aperture

**Holographic beam**
- Back aperture
- Axial resolution, $b \propto s$
Axial propagation: Temporal focusing

Originally used for wide field two-photon microscopy
D. Oron, E. Tal, Y. Silberberg, Optics Express (2005)

\[ S_{2PE} \propto \frac{I^2}{\tau \cdot f} \]


**Temporal focusing: the principle**

It works for ultra short pulses (depth of 3μm with τ = 10fs)

To relax this condition: tilted angle

To optimize off-axis dispersion: blazed grating
Axial resolution: Digital holography and Temporal focusing

E. Papagiakoumou et al. Optics Express (2008)
3D wave front shaping: GPC and Temporal focusing

Axial resolution with temporal focusing: Results

Gaussian beam

GPC+TF

Holographic beam+TF

Grating=830 l/mm
Obj =60x NA 0.9
f=500mm

OUTLINE

Optical techniques:

- Digital holography
- Generalized phase contrast method
- Temporal focusing
  
  Phase modulation

Results:

- 2P optogenetics in brain slices
- Propagation of shaped beams through scattering media
RESULTS: 2P Photoactivation of HEK 293 cells

ChR2-H134R-GFP transfected cultured HEK

$\lambda_{\text{exc}}$=850 nm; excitation density=0.45 mW / $\mu$m$^2$; 10ms pulse

Axial resolution

Lateral resolution

RESULTS: 2P Photoactivation in brain slices

Thy1-ChR2-YFP transgenic mice

Excitation = 0.3-0.5 mW/μm²; depth 60-70 μm, 10 ms

Action potential threshold around 10 μm

Firing frequency increases with spot size

E. Papagiakoumou et al., Nat. Methods (2010)
RESULTS. Multiple processes, multiple cells

$\lambda_{\text{Exc}} = 920 \text{ nm}$; Excitation = 0.6 mW/µm$^2$; pulse = 10 ms

$\lambda_{\text{Exc}} = 920 \text{ nm}$, Excitation = 0.3-0.5 mW/µm$^2$; pulse = 10 ms
CONCLUSIONS

• Phase modulation of optical wave fronts permit a 3D sculpting of the excitation volume:

  *Lateral light patterning:*
    *digital holography*
    *generalized phase contrast methods*

  *Axial resolution:*
    *temporal focusing*

• **TF:** to control axial resolution **AND** to maintain shaped patterns in scattering media
CONCLUSIONS:

Which is the optimal illumination method for optogenetics?

Young Frankenstein, M. Brook 1974
CONCLUSIONS: comparison

Table 1. Summary of the illumination techniques for 2P photoactivation

<table>
<thead>
<tr>
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CONCLUSIONS: comparison

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More Flexibility
Increased FOV
3D

D. Oron et al. PBR (2012)

More Flexibility
Increased FOV
3D

 이것이 표준적인 2P 이용에 대한 조명 기술의 요약입니다. 각 기술의 주요 특성은 다음과 같습니다:

- **Implementation**: 2P микроскоп + grating for TF, DH and grating for TF, 2P микроскоп + setup for GPC and grating for TF.
- **Max excitation density**: Laser power/w₀, Laser power/(FOV/4), Laser power/(FOV/4).
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- **Diffraction efficiency**: Constant, Constant, Constant.
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More Flexibility
Increased FOV
3D

D. Oron et al. PBR (2012)

More Flexibility
Increased FOV
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This is a summary of the illumination techniques for 2P photoactivation. The key characteristics of each technique are as follows:

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More Flexibility
Increased FOV
3D

D. Oron et al. PBR (2012)
THANK YOU!!

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