Optogenetics meets optical wavefront shaping

Shy Shoham

Optogenetic stimulation by ultrashort laser pulses could allow neural circuits in the living brain to be probed with cellular resolution, despite pervasive light scattering. Now sophisticated new multiphoton stimulation systems that strike a better balance between lateral and axial resolution help realize this potential by matching the illumination volume to the soma's dimensions.

Our vision relies on the interaction of light with cells in our nervous system, but it is only in the last few years that a cadre of 'optogenetic' light-gated proteins have emerged that allow researchers to methodically use photobiology to probe (stimulate or inhibit) neurons and other excitable cells in intact animals. Optogenetics has distinguished itself from other neurostimulation methods (mostly electrode-based) primarily through its ability to genetically target specific cell subpopulations. Many researchers are already expressing probes such as the algal light-activated cation channel Channelrhodopsin-2 (ChR2) in target cell populations in various brain regions and using implanted optical fibers or LEDs (for superficial structures) to drive the entire targeted population with millisecond-precision pulses of blue light[1]. Various probes expressed in distinct or overlapping populations can also be selectively triggered by using different wavelengths of light.

Optogenetics offers another path to selective targeting: light can easily be microscopically focused onto specific cells, and moreover, projected patterns of light can be used to selectively and flexibly control trains of action potentials distributed across entire populations of neurons. Optical targeting works well in optically accessible neural structures such as cell cultures, the retina and for neurons in transparent organisms like *Caenorhabditis elegans* and *Danio rerio*, but light is heavily scattered as it propagates through brain tissue (the characteristic scattering distance of blue light is only several tens of micrometers), and standard light projection methods cannot effectively target single neurons throughout a thin brain slice, let alone structures in the living brain.

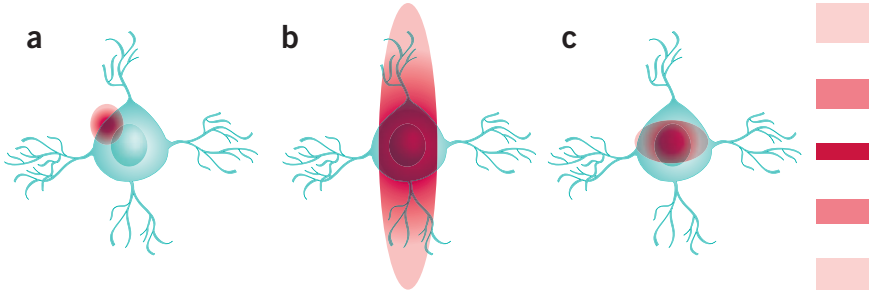

Figure 1 | The challenge of optogenetic multiphoton stimulation. (**a**) Ineffective stimulation with a high-numerical-aperture beam. (**b**) Stimulation with a low numerical aperture: the focal width matches a typical soma, but neurons outside the focal plane will also be excited. (**c**) A temporally focusing pulse (seen propagating on the right) is used for restricting the axial resolution, decoupling it from the lateral resolution.

Shy Shoham is in the Faculty of Biomedical Engineering, The Technion, Israel Institute of Technology, Haifa, Israel.
e-mail: sshoham@bm.technion.ac.il

To address this limitation, it was most natural to resort to the optical method most commonly used to microscopically image neural structures in the brain: multiphoton excitation. The extreme photon fluxes that occur when ultrashort pulses of light are tightly focused give rise to nonlinear optical effects, including the simultaneous absorption of pairs or even triplets of photons by a single chromophore. Multiphoton excitation of ChR2 is attractive because it replaces excitation by a single blue-light photon with two infrared photons that are much less prone to scattering, but even more importanly because it naturally discounts the effect of light scattering by overemphasizing the effect of 'ballistic' (unscattered) photons that arrive simultaneously at the focal spot.

However, researchers who rushed to stimulate ChR2-expressing cells using their two-photon microscopes quickly ran into a 'catch': the typical diffraction-limited focal volumes used to achieve the conditions of multiphoton excitation are so small—about 1/1,000 of the volume of a typical cell body—that only a tiny fraction of the available channels on the cell's outer membrane are excited under these conditions, yielding ineffective stimulation that cannot depolarize most cells to the action potential threshold (**Fig. 1a**). Trying to increase this focal volume by reducing the effective numerical aperture of the optical system does not solve the problem because the lateral and axial dimensions of the excited focal spot are coupled: a tenfold decrease in lateral resolution corresponds to a 100-fold loss of axial resolution, leading to an unacceptably deep, nonselective stimulation volume (**Fig. 1b**). A simple solution to this resolution versus effectiveness tradeoff is to move the tiny stimulation spot very rapidly around, integrating the cumulative effect of many locations, a strategy used last year by Rickgauer and Tank[2] for two-photon ChR2 neural stimulation with a 30-milisecond spiral scan. Similar approaches have been used for integrating over many tiny (extracellular) glutamate photolysis points[3,4] and for functional multiphoton (intracellular) calcium imaging[5].

Two studies, by Valentina Emiliani[6] and colleagues in this issue of *Nature Methods*, and by Aliphasa Vaziri and colleagues in the 29 June 2010 issue of the *Proceedings of the National Academy of Sciences of the United States of America*[7], present

methodological breakthroughs aimed at directly overcoming this challenge. Rather than rapidly scan a tiny (sub-micrometer) laser focus, both studies shift our attention to large stimulation spots on the order of ten micrometers that more closely match cellular dimensions (**Fig. 1c**).

In Vaziri's design[7], the authors removed the scan lens from a commercial two-photon microscope to obtain the 'widefield' illumination scheme (commonly used with incoherent light sources but rarely with lasers), whereas Emiliani's system[6] can be used to project complex light patterns using a sophisticated projection method called generalized phase contrast (GPC), developed by one of the study's coauthors, Jesper Glückstad[8]. Both studies resolve the fundamental coupling of axial and lateral resolution by incorporating a tilted diffraction grating into their respective optical systems to obtain strong 'temporal focusing', a clever trick for achieving axial sectioning in widefield multiphoton excitation, suggested 5 years ago by Yaron Silberberg and Dan Oron[9]. The diffraction grating splits the laser beam into spectral components that only meet in the focal plane. Outside the focal plane, the laser pulse is effectively longer or 'temporally defocused', and multiphoton excitation is far less likely to happen, even though the beam's diameter is almost unchanged.

How do the two systems generate light patterns? Vaziri's system[7] generates a fixed-size spot that can be sequentially directed at different neurons every few milliseconds using the microscope's scanning mirrors, whereas Emiliani's system[6] uses a scanless projector (GPC) that can flexibly switch patterns in video rate, and allowed the researchers to systematically explore the effect of changing the illumination spot diameter and to dynamically and simultaneously stimulate multiple dendrites and neurons present in the stimulation field. GPC essentially uses the same physical concepts that allow phase contrast microscopes to view completely transparent objects that only shift the light wavefront's phase, without absorbing it. In GPC projection, a shift in the light wavefront's phase is induced by miniature transparent liquid crystal displays called spatial light modulators (SLMs), converted to intensity by a phase-contrast filter and the resulting light pattern is used to excite the neurons. The same SLMs are also used to generate computer-generated holograms[10,11] (CGHs), another emerging photostimulation strategy in which input light is used efficiently even when the target pattern is sparse (covering a tiny fraction of the field), and in which multiple targets arbitrarily distributed in different planes in three dimensions can be illuminated. However, when CGHs are used to project the wide multipoint patches used in these studies, the illumination spot develops a characteristic high-frequency interference noise known as 'holographic speckle'[11] and becomes extremely nonuniform, a problem that GPC does not have.

How will the new methods be used? The new techniques are primarily intended for probing neural circuits in thin brain slices (where they were tested), and in accessible cortical structures *in vivo*. For example, they could be used to rapidly map functional connections or examine whether the correlated firing of multiple neurons causes nonlinear recruitment effects over the output of dendrites, muscles or over sensory perception. The single-spot system concentrates all of the available light exactly onto a single 'typical' cell, and will allow greater depth penetration and access to a larger stimulation field. The GPC method is less light-efficient for large stimulation fields but is more flexible: it can provide simultaneous (rather than sequential) access to different neurons, and also target dendrites, axons and synapses distributed in a two-dimensional region. As is, neither method will allow simultaneous optical stimulation of large groups of neurons distributed over large regions and/or in three dimensions, a task that may be best handled by the next generation of CGHs in which the light wavefront's phase and amplitude are both simultaneously shaped[12]. Nevertheless, these new contributions usher us into an exciting era in which the sophistication of the optical solutions being developed for experimental neuroscience matches the tremendous challenges that we face in attempting to observe, perturb and understand the highly distributed nervous system at the spatial and temporal scales in which it operates.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Zhang, F. *et al. Nat. Protoc.* **5**, 439–456 (2010).
2. Rickgauer, J. P. & Tank, D.W. *Proc. Natl. Acad. Sci. USA* **106**, 15025–15030 (2009).
3. Shoham, S., O'Connor, D.H., Sarkisov, D.V. & Wang, S.S. *Nat. Methods* **2**, 837–843 (2005).
4. Nikolenko, V., Poskanzer, K. E. & Yuste, R. *Nat. Methods* **4**, 943–950 (2007).
5. Grewe, B.F., Langer, D., Kasper, H., Kampa, B.M. & Helmchen, F. *Nat. Methods* **7**, 399–405 (2010).
6. Papagiakoumou, E. *et al. Nat. Methods* **7**, 848–854 (2010).
7. Andrasfalvy, B.K., Zemelman, B.V., Tang, J. & Vaziri, A. *Proc. Natl. Acad. Sci. USA* **107**, 11981–11986 (2010).
8. Glückstad, J. *Opt. Commun.* **130**, 225–230 (1996).
9. Oron, D., Tal, E. & Silberberg, Y. *Opt. Express* **13**, 1468–1476 (2005).
10. Lutz, C. *et al. Nat. Methods* **5**, 821–827 (2008).
11. Golan, L., Reutsky, I., Farah, N. & Shoham, S. *J. Neural Eng.* **6**, 066004 (2009).
12. Jesacher, A., Maurer, C., Schwaighofer, A., Bernet, S. & Ritsch-Marte, M. *Opt. Express* **16**, 2597–2603 (2008).