polarization, also participates in the LTS in TC cells, particularly during sleep spindles. The effects of its distribution within the TC cell could also be studied with compartmental models, in conjunction with the distribution of the T-currents. The optimal distributions of these currents may not be independent of each other, so they need to be jointly varied.

The calcium that enters the neuron during an LTS must be internally bound or extruded to maintain the equilibrium of free calcium in the cell in the long term. This is accomplished by Ca^{2+}/Na^{+} metabolic exchangers in the plasma membrane. Reducing the number of T-channels needed to trigger an LTS would reduce the number of calcium ions that need to be extruded later, and hence would reduce the energy that the TC cell must expend to function. In the hippocampus, nonmyelinated axons have a fast sodium current and delayed potassium current, which reduces the overlap of the currents and minimizes the cost of an action potential (Alle et al., 2009). Similarly, pyramidal neurons and fast-spiking interneurons in the cerebral cortex also minimize energy expenditure for the patterns of action potentials they generate *in vivo* (Hasenstaub et al. 2009). This may be a general principle for neural information processing systems (Laughlin et al., 1998; Laughlin and Sejnowski, 2003).

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A new phase for two-photon microscopy

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A commentary on

SLM microscopy: scanless two-photon imaging and photostimulation using spatial light modulators

by Volodymyr Nikolenko, Alan Woodruff, Brendon Watson, Rafael Yuste, Darcy Peterka and Roberto Araya

Two-photon excitation has enabled remarkable advances in our understanding of the nervous system. Its three-dimensional resolution, superior depth penetration, and minimal phototoxicity for out-of-focus portions of the sample have made it the technique of choice for an impressive array of applications. For all of its advantages, however, it is not without its limitations; for neuroscience, perhaps chief among these is the time it takes to collect a large image over a field of view containing many neurons. To address this limitation, Nikolenko et al. (2008) have provided a new method for creating customized two-photon excitation, thereby allowing the user to conduct photostimulation or imaging from tens of neurons simultaneously.

Conventional two-photon microscopy assembles an image by scanning a single laser spot over the sample. While there are fast-scanning microscopes that can collect an entire frame in 1/30 of a second, these instruments are hampered by high noise levels. The dominant source of noise is not the instrument itself: instead, it arises from the limited number of photons that are collected in each pixel. Reducing the noise simply requires more photons—but under typical conditions, each illuminated fluorophore emits photons at its maximal rate, which means that the only way to get more photons is to integrate over longer times.

Efforts to circumvent this limit have mostly taken one of two approaches (see Conchello and Lichtman, 2005; Wilt et al., 2009 for reviews). Some instruments illuminate many pixels simultaneously, satisfying the integration time for many pixels in parallel. The others carefully choose a restricted set of "interesting" pixels, thereby reducing the total number of pixels that need to be acquired during each scan.

Nikolenko et al. present an elegant technique, which they call "SLM microscopy," that combines both of these strategies into a single system. Their system targets two-photon excitation simultaneously to up to several tens of points within the field of view. The authors achieve this feat using a spatial light modulator (SLM), which alters the oscillation *phase* of the incoming light. A shift in phase does not in itself alter the intensity of the light; however, because of constructive and destructive interference, a change in phase can lead, upon propagation, to a dramatic re-distribution of intensity. For example, a microscope objective can be thought of as a device that simply adds a spatially-varying phase to the incident illumination; constructive and destructive interference then causes the phenomenon that we usually think of as focusing to a diffraction-limited point.

Nikolenko et al. (and related work Lutz et al., 2008; Papagiakoumou et al., 2008), following in the footsteps of holography pioneer Gabor (1948), deliberately perturb the phase of the input light to the objective. The result, of course, is a microscope that no longer focuses all the incoming light to a single, diffraction-limited spot. Ordinarily, this would not be taken as a step forward. However, the SLM consists of more than a million individually-addressable elements, and thus allows phase to be precisely manipulated in nearly arbitrary spatial patterns. The authors employed a computational algorithm to calculate a phase pattern that, after passage through the objective, illuminated many distinct spots. Crucially, the position of these spots is under the control of the user, and thereby allows one to direct light to many specific targets, even ones that are above or below the objective's plane of focus. Rather than the usual photomultiplier tube used when scanning a single point, the emitted light is focused onto a CCD camera, which preserves spatial information about the emission source.

Nikolenko et al. demonstrated SLM microscopy's utility with two applications. The first is photostimuluation with caged glutamate. The authors were able to simultaneously stimulate multiple spines on the same neuron, or multiple neurons simultaneously. The high power needed for two-photon uncaging currently acts as a barrier to selecting more than a modest number of spots. Nevertheless, the ability to stimulate multiple spots seems likely to yield advances in our understanding of both single-cell membrane properties and neuronal circuits.

The second application was to image activity, via a calcium indicator, simultaneously in multiple neurons at speeds ranging from 15-60 Hz. The resulting signals were sufficient to clearly detect transients due to single action potentials. Several methods (reviewed in Ji et al., 2008; Wilt et al., 2009) for fast imaging of neuronal activity have previously been developed, each with their own advantages. A strength of SLM microscopy, because it acquires several regions in parallel, is its ability to achieve relatively low noise levels (at least by the standards of some other two-photon techniques) from the selected targets, even at high speeds.

Over the past few decades, microscopy – one of the oldest pillars of science – has undergone an extensive era of new development. SLM microscopy exploits our burgeoning ability to shape light to our needs, and is a welcome addition to the overall progress in optics and neuroscience.

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Sharing with Python

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A commentary on

Python for large-scale electrophysiology

by Martin Spacek, Tim Blanche and Nicholas Swindale

Researchers changing scientific fields are often surprised to discover how different the cultures in their new and old fields are. While the neuroscience culture is vibrant and stimulating in many ways, neuroscience is not a field with the strongest tradition for sharing or division of labor. In physics there has effectively been a division of labor between experimentalists and modelers for about 100 years. It was realized that it is simply too difficult for a single person or a single research group to mas-