Enlightened neuroscience

Neuroscience methods are undergoing a dramatic change owing to improvements in optical probes, but standardized evaluation procedures would aid probe development and uptake.

In the nineteenth century, light microscopy coupled with cellular labels provided insights into neuronal organization that were critical for establishing the neuron doctrine—a central tenet of modern neuroscience. Despite the continued importance of microscopy, the intervening years have seen electrophysiology and magnetic resonance imaging (MRI) take the starring role in neuroscience. But recent advances in microscopy, fluorescent-probe design and light-based methods of active neuronal control are providing neuroscientists with powerful new optical methods.

The slow rise of optical methods in neuroscience started in the 1970s and 1980s with the development of fluorescence-based voltage and calcium sensors capable of detecting neuronal electrical spiking activity or the accompanying increase in intracellular calcium. These sensors had the potential to enable imaging of neuronal signaling with cellular and subcellular resolution in many neurons simultaneously, something that is impossible with electrophysiology or MRI. Unfortunately, delivery and performance limitations kept these sensors from fulfilling their potential. The development of genetically encoded calcium and voltage indicators promised to overcome the problems of delivery, but inadequate signals of early versions, particularly *in vivo*, continued to hamper progress.

With the recent arrival of several newly available optical tools, the field is undergoing a transformation, and neuroscientists' use of these tools appears to be on the verge of explosive growth. The developments in the field and accompanying excitement were highlighted in October by reviews in an Insight on neurotechniques in *Nature* and a special issue of *Science* that highlighted neuroscience methods.

In this issue of *Nature Methods*, three papers describe new developments in this area. Looger and colleagues use structure-guided mutagenesis to engineer an improved member of the GCaMP family of genetically encoded calcium sensors on page 875. Meanwhile, on page 883, Lagnado and colleagues fuse an earlier GCaMP sensor to synaptophysin to target it to presynaptic boutons. Both sensors display larger signal-to-noise ratios and improved linear responses to increasing spike rate. Finally, on page 891, Ramanathan and colleagues combine the use of light-sensitive ion channels for neuronal stimulation—a technique that exploded onto the scene in the last few years—with GCaMP-based neuronal recording, to demonstrate the first fully genetically encoded light-based electrophysiology.

These optical tools have reached the point at which they can start fulfilling their promise, but improvements are still needed before they approach their full potential. Genetically encoded calcium sensors still cannot detect single spikes with 100% reliability, which restricts their usefulness in brain regions with sparse spiking events. In contrast, regions with very high frequency spiking need sensors with faster kinetics. Voltage sensors, meanwhile, need to see many-fold improvements in performance, primarily in signal-to-noise ratio, before they will be on par with calcium sensors. No single sensor in each class will fulfill all requirements and different sensors with specific properties will be needed for different experimental systems. Comparative evaluations are therefore critical.

Unfortunately, the characterization and assessment of new probes is often hindered by a lack of experimental standards. For example, binding affinities of calcium sensors are usually determined in nonphysiological buffers that lack magnesium. Consequently, the results often are not indicative of the affinity *in vivo*. Though it may be useful to use 'historical' buffers for comparison to old data, it is important that the community also begin to use more physiological buffers.

But ultimately the performance of a new sensor or other tool can only be determined by direct comparison to existing tools and by application in realistic experimental systems. Looger and colleagues directly compared their sensor to two existing state-of-the-art sensors in quantitative brain slice experiments and took the step of not only testing the performance *in vitro*, in cell culture and brain slices, but also *in vivo* in the worm, fruit fly and mouse. As a result, potential users have a good idea of what to expect from this sensor in their system and application of choice.

Such extensive testing is often only feasible for research groups in large centers. The establishment of consortia devoted to evaluating new tools would help enormously by providing uniform evaluations. In the absence of a centralized process for comparative evaluation, direct comparison to existing tools in a standard experimental preparation such as neuronal cell or slice culture with naturalistic action potentials is crucial. Long-term *in vivo* expression of protein-based probes should also be part of any standard evaluation to test for deterioration of neuron or probe function.

The general need for standards in evaluating fluorescent probes was a conclusion of the recent Janelia Conference on Fluorescent Protein and Biological Sensors. Rapid adoption of appropriate standards by the community would help ensure that the enormous potential of these tools is realized in the shortest time possible.