METHODS IN BRIEF

NEUROSCIENCE

The ins and outs of optogenetic silencers

Silencing neurons using light-driven pumps is known to be specific, fast and effective, but the consequences of silencing on neuron physiology remain under investigation. Raimondo *et al.* tested the effect of two of the most common optogenetic silencers—the light-driven outward proton pump Arch and inward Cl^- pump NpHR3.0—on cells after the light-activation period. They monitored the electrophysiological properties of hippocampal neurons in rat brain slices before and after light-induced silencing, and they found that activation of NpHR3.0 increased the probability of spiking after photoactivation in the silenced cells. This effect was not observed using Arch and is thought to be due to the fact that high Cl^- currents alter GABAergic transmission in these cells. These findings might help with the design of experiments that use these optogenetic tools.

Raimondo, J.V. *et al. Nat. Neurosci.* advance online publication (24 June 2012).

GENE EXPRESSION

Quantifying single transcripts

RNA binding proteins can be used to image single RNA transcripts in living cells. In this approach, a phage coat protein (CP) is fused to a fluorescent protein (FP) and binds a transcript engineered to be recognized by the CP-FP fusion. Quantitative use of this approach remains challenging, however. In recent work, Wu *et al.* addressed some of the challenges. They reduced background from unbound CP-FP fusions, which bind to the target RNA as dimers, by constructing single-chain tandem dimers for two phage systems. They used fluorescence fluctuation spectroscopy to obtain brightness and diffusion information on these constructs in cells expressing the target RNA, thus yielding copy numbers for all diffusing species. Fluorescence fluctuation spectroscopy permits the quantitative study of engineered exogenous and endogenous transcripts in living cells.

Wu, B. *et al. Biophys. J.* **102**, 2936–2944 (2012).

MICROSCOPY

Toward deep-tissue fluorescence imaging

The scattering of light in tissues is the bane of many researchers who would like to image biological events deep under the surface. However, scattered light also contains information about the sample that can be exploited. Wang *et al.* capitalize on this with a technique to extend focused fluorescence imaging to a depth of 2.5 millimeters. A focused ultrasound pulse is applied to a scattered beam of light deep within the sample, which causes a portion of the light to become frequency shifted. This frequency-shifted light is phase conjugated with high gain using a digital optical phase conjugation scheme. The phase conjugate beam is reflected back through the sample and converges at the original location of the ultrasound focus, thereby resulting in an optical focus deep within the tissue. Though the method has so far been applied only in proof-of-principle experiments, it holds promise for deep-tissue fluorescence imaging.

Wang, Y.M. *et al. Nat. Commun.* **3**, 928 (2012).

STEM CELLS

Neural progenitors from fibroblasts

Cocktails of transcription factors have been used to reprogram fibroblasts to become neurons and neural progenitor–like cells in the mouse. Neural progenitor cells have also been generated by differentiating pluripotent stem cells (both the embryonic and induced pluripotent varieties). In a new twist to the reprogramming tale, Ring *et al.* now report reprogramming of both mouse and human fibroblasts directly to neural progenitor–like cells using a single transcription factor, Sox2. Under the appropriate culture conditions, Sox2 transduction yields so-called induced neural stem cells. These cells show the expected gene expression profiles; renew in culture; generate neurons, astrocytes and oligodendrocytes; and do not form teratomas upon transplant into the mouse brain.

Ring, K.L. *et al. Cell Stem Cell* **11**, 100–109 (2012).