METHODS IN BRIEF

MICROSCOPY

STED with twice the depletion

In stimulated emission depletion (STED) microscopy, a powerful super-resolution microscopy approach, an excitation laser and a donut-shaped STED laser are scanned together over a field of view. The excitation laser excites all fluorophores in an area, and the STED laser sends fluorophores into a dark state, leaving only the center of the donut fluorescent. Gao *et al*. have developed double-depletion STED, or STEDD. In STEDD, artificial background intensity is removed by the addition of a second STED pulse to conventional STED; this second, delayed pulse specifically depletes the fluorescent center of the donut, leaving only background. By using time-resolved detection, the researchers removed background, thus improving their super-resolved images. They demonstrated improved resolution when imaging fluorescent beads as well as labeled microtubules in mammalian cells.

Gao, P. *et al*. *Nat. Photonics* **11**, 163–169 (2017).

CHEMISTRY

Solution-state ^{13}C NMR gets a boost

Nuclear magnetic resonance (NMR) spectroscopy is widely used in organic chemistry, metabolomics and structural biology research, as well as for *in vivo* imaging, but this technique suffers from low sensitivity. Liu *et al*. describe an approach that boosts the sensitivity of solution-state ^{13}C NMR by 1,000-fold via the application of dynamic nuclear polarization (DNP) using nitroxide radical polarizers. Although DNP is very often used in solid-state NMR applications to improve signal-to-noise ratios, it has been a challenge to implement DNP in combination with high-magnetic-field solution-state ^{13}C NMR studies. The new method enabled the authors to analyze biologically relevant compounds, including metabolites, at room temperature and with greatly enhanced sensitivity. The DNP technique may yet prove to be a general approach to increase the sensitivity of solution-state NMR.

Liu, G. *et al*. *Nat. Chem*. http://dx.doi.org/10.1038/nchem.2723 (2017).

NEUROSCIENCE

Optogenetic feedback in real time

Optogenetics uses light to control the activity of neurons, but reading out neural activity and using the obtained information to provide rapid feedback for continued optogenetic control is challenging. Prsa *et al*. imaged GCaMP6f-labeled neurons in the mouse motor cortex with two-photon microscopy and transformed the information from this activity sensor, in real time, into optogenetic stimulation pulses in the corresponding somatosensory cortex. When the rate of stimulation exceeded a certain threshold, a water reward was delivered to the mice. The researchers show that mice learned to control the activity of single neurons or combinations of neurons in the motor cortex to increase their reward. They suggest that their technology could pave the way for more efficient brain–machine interfaces, and also could help scientists dissect behavioral circuits.

Prsa, M. *et al*. *Neuron* **93**, 929–939 (2017).

MICROSCOPY

Imaging fast subcellular dynamics with light sheets

Light-sheet microscopy is a valuable tool for imaging volumes at high speeds. However, some cellular processes occur too rapidly to be imaged, even with state-of-the-art equipment. Dean *et al*. developed parallelized light-sheet fluorescence microscopy (pLSFM) for rapid 3D imaging. In pLSFM, three laterally and axially displaced Gaussian light sheets are used to illuminate a tilted, coverslip-mounted specimen. The arrangement of the light sheets allows fluorescence from each light sheet to be detected independently, thus enabling high-speed volume imaging of samples such as cells grown on a coverslip. The researchers observed a nearly threefold increase in imaging rate relative to that of conventional light-sheet microscopy, with no change in photobleaching. They also show that the method enables rapidly moving endosomes and propagating calcium waves to be tracked in individual neurons.

Dean, K.M. *et al*. *Optica* **4**, 263–271 (2017).