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Shining light on neural circuits in mice

Optogenetic manipulation in behaving animals requires lightweight light sources that have minimal effects on the behavior of the animals. Poon and colleagues have developed light sources that weigh less than 50 mg and are small enough to be fully implanted. A radiofrequency power source controls and powers these devices remotely and wirelessly, allowing for optogenetic stimulation without weighing the animals down. Depending on the design of the devices, they can be implanted into the brain, near the spinal cord or close to peripheral neurons in the extremities. The researchers have used these devices to modulate walking behavior, pain perception and immediate early gene expression in channelrhodopsin 2–expressing mice.

Article p969

Designing sgRNAs for in vivo screens

The rise of the CRISPR-Cas9 system for genome editing has gone hand in hand with the effort to design single guide RNAs (sgRNAs) with uniformly high activity. Screens for the most active sgRNAs are usually performed in cells, but Giraldez and colleagues’ goal was to investigate the principles underlying efficient targeting in vivo. They targeted each of 128 genes in the zebrafish embryo with ten sgRNAs and determined the effect of sequence composition on stability and activity. They found that sgRNAs truncated by one or two nucleotides or those with 5’ mismatches still retained high activity, increasing the number of target sites in the zebrafish genome eightfold. The researchers integrated their observations into a predictive model called CRISPRscan that searches for sgRNAs on genes of interest and scores their activity.

Article p982

Spectrally resolved STORM

Highly multiplexed super-resolution imaging remains a challenge because of the limited number of dyes of different colors suitable for techniques such as photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). Xu and colleagues overcame this issue by combining single-molecule spectroscopy with STORM. Their method, spectrally resolved STORM (SR-STORM), uses a wide-field scheme to measure the emission spectra of stochastically activated fluorophores and results in rapid ‘true-color’ super-resolution imaging of multiple subcellular components. SR-STORM was demonstrated for simultaneous imaging of subcellular structures labeled with four highly overlapping dyes in three dimensions.

Brief Communication p935

Engineering fast-switching RNA devices

RNA-based regulatory devices, such as ligand-binding aptamers fused to hammerhead ribozyme, are invaluable for modulating the activity of a target transcript and have been shown to work in different organisms. These devices are fused to targets, and upon ligand binding, self-cleavage of the ribozyme is blocked, allowing expression of the target. Current ribozyme designs are based on the switching of secondary structures upon ligand binding and show relatively slow kinetics. To overcome this limitation, Smolke and colleagues developed a flow cytometry– and sequence-based high-throughput screen for RNA device libraries that relies on the tertiary interactions between aptamers and their ligands. They successfully isolated RNA devices for various ligands with high dynamic ranges and sensitivity. Insights gained will also aid in the design of new RNA devices.

Article p989

Inferring cell-cycle state from fixed images

Differences in cell-cycle position can be a major source of cell-to-cell variability among unsynchronized cells. However, separating the effects of cell-cycle position from other experimental variables when investigating factors such as protein abundance requires the determination of both an individual cell’s position in the cell cycle and the protein abundance in that cell. Such measurements are technically challenging. For this reason, Pe’er, Pelkmans, Liberali and colleagues developed a method, called Cycler, to infer trajectories of cell-cycle progression from fixed cell populations. Cycler starts with static images of unperturbed cells and generates single-cell measurements of multiple features to ultimately generate a cell-cycle trajectory, in which cells are ordered according to their relative positions in the cell cycle. By studying the abundances of several proteins throughout the cell cycle, the authors demonstrate that Cycler is a powerful tool for quantifying sources of cell-to-cell variability.

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