METHODS IN BRIEF

CELL BIOLOGY

Optogenetic positioning of organelles

The function of organelles such as the endoplasmic reticulum is tightly linked to their position, but positioning at defined sites may not be necessary for other organelles. van Bergeijk *et al.* present a method to localize organelles in a light-dependent fashion, making it possible to address the function and requirement of organelle positioning. The researchers adapted the TULIP (tunable, light-controlled interacting protein tags) system to recruit peroxisomes, mitochondria or endosomes to molecular motors such as kinesin, dynein or myosin. This allowed them to target these organelles to the cell periphery, cell center or dendritic spines. Using this approach, the researchers demonstrated a role for endosomes in the regulation of growth-cone dynamics.

van Bergeijk, P. *et al. Nature* **518**, 111–114 (2015).

IMAGING

Probing molecular stoichiometry at super-resolution

Super-resolution imaging techniques such as photoactivated localization microscopy (PALM) are becoming widely used in biology. However, determining protein-complex stoichiometry using PALM remains challenging, in part because of blinking associated with fluorescent proteins. Rollins *et al.* developed a stochastic approach to measure stoichiometry using PALM data, based on continuous-time aggregated Markov model techniques developed to study ion channels. This approach models fluorescent protein activation, blinking and photobleaching to determine the number of fluorophores in a complex. Using simulated and experimental data, the researchers benchmarked their method and demonstrated its utility in deriving the number of fluorophores in a complex and the error associated with that estimate. The method provides a promising new approach to solving the molecular counting problem in super-resolution microscopy.

Rollins, G.C. *et al. Proc. Natl. Acad. Sci. USA* **112**, E110–E118 (2015).

SEQUENCING

The genome and transcriptome of a single cell

Single-cell sequencing enables the study of tissue heterogeneity and rare cells, but it has been impossible to directly connect genotype with gene expression from such data. Dey *et al.* now describe genomic DNA–mRNA sequencing (DR-Seq), a method to obtain both types of information from the same cell. In DR-Seq, the contents of a single lysed cell are subjected to linear RNA amplification based on the CEL-Seq (cell expression by linear amplification and sequencing) protocol and quasilinear amplification of DNA based on the MALBAC (multiple annealing and looping-based amplification cycles) protocol. The DNA and RNA templates are not separated before amplification, thereby reducing the chances of contamination and material loss. The researchers applied DR-Seq to mouse embryonic stem cells and a breast cancer cell line, finding that high gene copy numbers may increase gene expression variability between cells.

Dey, S.S. *et al. Nat. Biotechnol.* doi:10.1038/nbt.3129 (19 January 2015).

MICROSCOPY

Closing in on video-rate STED nanoscopy

Imaging large fields of view using stimulated emission depletion (STED) microscopy with single-point scanning requires long imaging times, which limits the utility of this approach for studying rapid biological processes. Recent efforts have sought to increase the speed of STED imaging through massively parallelized acquisition. Bergermann *et al.* expanded on an existing approach to achieve 2,000-fold parallelization of STED in two colors. In this approach, wide-field excitation is combined with patterned off-switching to generate the confined fluorescence that is the hallmark of STED. Compared to previous attempts for massively parallelized STED, this approach was able to achieve the same resolution as single-point scanning STED while imaging a 30-fold-larger field of view. These breakthroughs now make camera frame rates the limiting factor in acquisition speed and pave the way for imaging at high spatiotemporal resolution.

Bergermann, F. *et al. Opt. Express* **23**, 211–223 (2015).