METHODS IN BRIEF

GENOMICS

Single-organelle sequencing

Organellar genomes can exhibit genetic variation, even within cells, with consequences for disease penetrance and organellar inheritance. Although attempts have been made to sequence DNA at single-organelle resolution, they have been limited to short genomic regions. Morris *et al*. now develop an approach to sequence the whole genome of individual mitochondria by isolating single organelles via micropipette, amplifying the full genome using specific primers and nested or seminested PCR, and then carrying out sequencing library construction. Using the technique, the researchers assessed the mitochondrial genomes of mouse neurons and astrocytes, and they found high levels of single-nucleotide variation at multiple loci across individuals, across cells and within cells. Mitochondria of human neurons in primary culture appeared to have slightly lower variation, though further sampling is needed to establish this.

Morris, J. *et al*. *Cell Rep*. **21**, 2706–2713 (2017).

NEUROSCIENCE

Myelin quantification at nanoscale

Myelin tightly wraps around axons and forms an insulating sheath, which enables fast propagation of action potentials. To quantify the degree of myelination and the thickness of the underlying axon, Kwon *et al*. harness the reflective properties of the multilayered myelin sheath in a label-free and high-resolution imaging approach. The different layers in the myelin sheath have different refractive indices, and therefore incident light is partially reflected at the interphases between layers. The reflections from different interphases interfere with each other, and this information can be used to describe the properties of the myelin sheath. Kwon *et al*. show that they can determine the degree of myelination, the axonal diameter as well as swelling of myelin with their approach. The researchers applied the technology to assess the changes in myelin after a traumatic brain injury in mice.

Kwon, J. *et al*. *Nat. Commun*. **8**, 1832 (2017).

CHEMICAL BIOLOGY

Assessing protein activity in single cells

Measuring the activity of proteins using chemical probes and mass spectrometry requires sufficient amounts of the sample, is limited to abundant proteins and does not preserve cellular resolution. In a twist of the well-established proximity ligation assay, Li *et al*. measure the activity of proteins of interest in single cells. Their activity-dependent proximity ligation (ADPL) assay relies on tagging of active enzymes with chemical probes that are specific for protein families such as serine hydrolases. The researchers then apply antibodies specific to the chemical probe and the protein of interest, followed by a proximity ligation assay. This technology allows them to visualize even low-abundance proteins in single cells. The researchers used the ADPL assay to measure the differential activity of a cholesterol ester hydrolase in different cancer cell lines and in patient tissue samples.

Li, G. *et al*. *Nat. Commun*. **8**, 1775 (2017).

IMAGING

Single-molecule imaging and force spectroscopy at extended depth

Single-molecule force spectroscopy is often combined with optical imaging approaches. Total internal reflection fluorescence (TIRF) microscopy and confocal microscopy provide high signal-to-noise ratios. However, imaging beyond a single focal plane is difficult, as TIRF microscopy is limited in imaging depth, while in confocal microscopy, movement of the sample stage or of the objective interferes with force measurements. Chang *et al*. introduce an electrically tunable lens (ETL) into the imaging arm of their optical tweezer setup. The focal length of the ETL could be changed at millisecond temporal resolution while extending the depth range to tens of micrometers. The researchers used their system to visualize the axial motion of fluorescently labeled epidermal growth factor receptors that were optically trapped.

Chang, M. *et al*. *Opt. Express* **25**, 32189–32197 (2017).