METHODS IN BRIEF

GENE EXPRESSION

Thousands of cell droplets

Two new studies scale up single-cell profiling by using microscopic droplets in an oil emulsion to encapsulate many thousands of cells for RNA sequencing library preparation. Klein *et al.* developed inDrop (indexing droplets), in which a library of barcoded DNA oligonucleotide primers reversibly linked to hydrogel in microspheres are coencapsulated individually with single cells. The researchers profiled over 10,000 mouse embryonic stem cells before and after withdrawal of leukemia inhibitory factor and uncovered rare subpopulations. Macosko *et al.* present Drop-seq, in which barcoded primers attached to beads are coencapsulated individually with single cells and then library preparation and sequencing in bulk are performed. The team used it to profile cell-cycle states of single cells and to characterize heterogeneity among nearly 45,000 mouse retinal cells.

Klein, A.M. *et al. Cell* **161**, 1187–1201 (2015).

Macosko, E.Z. *et al. Cell* **161**, 1202–1214 (2015).

PROTEOMICS

Ultra-mass-tolerant database searching

Mass spectrometry approaches are now sensitive enough to profile more than 10,000 proteins in a single experiment, yet the majority of tandem mass spectra generated in a proteomics experiment are still not matched to a peptide using database search algorithms. Many of these unmatched spectra are likely to represent post-translationally modified peptides or amino acid variants. Chick *et al.* reasoned that the very high mass accuracy of current mass spectrometry technology should allow these unmatched spectra to be assigned using ultratolerant database searching, allowing for variable masses of hundreds of different peptide modifications. They demonstrated their approach using the popular Sequest tool with a wide mass tolerance search to assign mass spectra from HEK293 cells. They were able to identify an additional 184,000 modified peptides representing hundreds of different modifications, including several rare modifications and amino acid variants.

Chick, J.M. *et al. Nat. Biotechnol.* **33**, 743–749 (2015).

CELL BIOLOGY

Mapping accessible chromatin in single cells

Methods that allow the analysis of a single cell's genome or transcriptome are invaluable for characterizing the heterogeneity and plasticity within cell populations. A method to profile chromatin accessibility now joins the single-cell analysis toolbox. Buenrostro *et al.*, who developed ATAC-seq (assay for transposon-accessible chromatin) for the rapid mapping of genome regulatory regions, adapt the technique using a commercial microfluidic device to allow chromatin accessibility profiling in single cells. The authors report that variation in the accessibility of regions bound by certain regulatory factors differs between cell types, and this information can be used to deconvolve cellular mixtures. Variations in single-genome accessibility also correlates with chromosome conformation, indicating that single-cell ATAC-seq can capture three-dimensional genome structure *de novo*.

Buenrostro, J.D. *et al. Nature* doi:10.1038/nature14590 (17 June 2015).

MICROSCOPY

Axially swept light-sheet microscopy

Light-sheet microscopy is a popular tool for biological imaging that offers advantages such as low photodamage, good optical sectioning and high speed. However, owing to out-of-focus light, single-photon light-sheet microscopes face a trade-off between *z* resolution and the size of the field of view, meaning that only small regions can be imaged with diffraction-limited *z* resolution. Dean *et al.* overcome this issue using a new light microscopy setup in which excitation light is swept through the sample along the *y* axis, yielding a thin strip of in-focus fluorescence surrounded by a blur. So that only the in-focus light is captured, a corresponding subset of pixels in the camera is made active in synchrony with the excitation light. The method yielded isotropic 390-nanometer resolution over a large volume and was used for three-dimensional tracking of clathrin-coated pits.

Dean, K.M. *et al. Biophys. J.* **108**, 2807–2815 (2015).