METHODS IN BRIEF

MICROSCOPY

Deeper quantitative phase imaging

Quantitative phase imaging (QPI) is a label-free imaging approach that offers advantages over conventional phase and differential interference contrast (DIC) imaging for quantitative analyses, such as measuring the local thickness and refractive index of the imaged structure. However, QPI does not work well for thick specimens, because multiple scattering caused by the specimen results in an incoherent background that degrades image contrast. Nguyen *et al.* addressed this problem by developing gradient light interference microscopy (GLIM). GLIM combines DIC microscopy with low-coherence interferometry and holography; this combination leads to rejection of much of the incoherent background, which results in high-contrast images of thick specimens. To demonstrate their approach, the researchers carried out 3D imaging of bovine embryos during early development and showed that GLIM could be used to assay embryo viability.

Nguyen, T.H. *et al. Nat. Commun.* **8**, 210 (2017).

GENETICS

A human tRNA atlas

tRNAs are one of the classic models for noncoding RNAs. Decades of research have explored their role in translation; more recently, however, this role has expanded to include other regulatory functions. To understand the scope of these functions, Gogakos *et al.* developed hydro-tRNAseq to annotate all tRNA genes. The authors subjected a 60–100-nt fraction of total RNA to limited alkaline hydrolysis to remove structure and modifications and thus created a cDNA library with substantially increased tRNA levels. Iterative mapping provided the researchers with a comprehensive map of tRNA genes. To refine the annotation, they combined the data with photo-activatable crosslinking and immunoprecipitation (PAR-CLIP) of a protein involved in pre-tRNA processing. The authors present a human tRNA atlas that includes tRNA expression levels.

Gogakos, T. *et al. Cell Rep.* **20**, 1463–1475 (2017).

IMAGING

Quantitative clearing-enabled tissue analysis

The function of a tissue depends on the distribution, identity and state of different cells that form the tissue. Analyzing the cellular organization in 3D can therefore help researchers understand complex tissues. Li *et al.* combined clearing-enhanced 3D microscopy with histocytometry to analyze tissue composition in mouse lymph nodes and other tissues. The researchers developed an optimized tissue-clearing protocol that maintained fluorescence from reporter proteins, that was compatible with immunolabeling and that retained cellular morphology. When combining their tissue-clearing approach with image analysis algorithms and cytometry tools, the researchers could quantify the distribution of B and T cells, macrophages and other cell types in mouse lymph nodes. As the clearing protocol is applicable to other tissues, the general strategy could be used to quantify cellular organization in other tissues as well.

Li, W. *et al. Proc. Natl. Acad. Sci. USA* **114**, E7321–E7330 (2017).

MASS SPECTROMETRY

An assessment of SWATH reproducibility

SWATH is a data-independent acquisition-based mass spectrometry (MS) technique for detecting and quantifying peptides of interest in a targeted fashion. This and other data-independent acquisition techniques are growing in popularity in proteomics, as they are not subject to the same stochastic sampling issues as traditional data-dependent acquisition-based MS. Collins *et al.* recently performed a large-scale study to assess the reproducibility of SWATH-MS in 11 labs around the world. Each lab evaluated an identical sample consisting of stable-isotope-labeled standard peptides spiked into an HEK293 cell digest. Each lab also acquired MS data in equivalent ways: i.e., they used the same instrument and same data acquisition parameters. The authors found that the data produced in the different labs were both qualitatively and quantitatively similar, and all labs consistently detected and quantified more than 4,000 proteins with good sensitivity and dynamic range.

Collins, B.C. *et al. Nat. Commun.* **8**, 291 (2017).