METHODS IN BRIEF

MICROSCOPY

Light-sheet microscopy on AutoPilot

Light-sheet microscopy has proven a versatile tool for imaging the behavior and development of numerous model organisms. However, image quality in light-sheet microscopy is affected by the orientation and optical properties of the specimen, which in the case of living samples can change over time. Royer *et al.* sought to automate acquisition of optimal images of living specimens. To do so, they developed a method for adaptive imaging called AutoPilot that integrates a multiview light-sheet microscope with movable light-sheet and detection planes through a computational approach capable of optimizing spatial resolution in real time. The AutoPilot framework allowed the team to carry out extended adaptive imaging of developing zebrafish and *Drosophila* embryos as well as whole-brain imaging of calcium signaling in larval zebrafish.

Royer, L.A. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3708 (2016).

NEUROSCIENCE

High-speed imaging of neural activity in behaving animals

Random-access microscopy with acousto-optic lenses operates at a speed of tens of kilohertz and is therefore ideally suited for measuring neural activity. However, random-access microscopy involves jumping between measurement points, which can easily lead to motion artifacts in behaving animals. Szalay *et al.* and Nadella *et al.* independently came up with similar strategies to overcome this limitation. Both research groups developed acousto-optic lens microscopes that are capable of line scanning at high speed. This makes it possible to scan small areas or volumes covering cell bodies or other regions of neurons and to implement motion-correction algorithms, enabling high-speed acquisition of neural activity in behaving animals. Both research groups apply their approaches in mice expressing GCaMP6 in their brains.

Szalay, G. *et al. Neuron* **92**, 723–738 (2016); Nadella, K.M.N.S. *et al. Nat. Methods* **13**, 1001–1004 (2016).

MOLECULAR BIOLOGY

Small RNAs from small samples

As the sequencing of single-cell transcriptomes takes off, many classes of RNA have been left behind. Nearly all protocols rely on poly-dT to prime the production of cDNA, which misses nonadenylated species, including microRNA (miRNA), small nucleolar RNA (snoRNA) and tRNA. A protocol from Faridani *et al.* addresses this gap by ligating 5' and 3' adaptors to all RNA species and suppressing amplification of the dominant rRNA fraction with masking oligos; unique barcodes also reduce amplification bias in this protocol. The researchers focused computational analysis on short species, finding thousands of miRNAs, tRNA fragments and snoRNA fragments, on average, in a single cell. They identified small RNAs that were differentially expressed between naive and primed human embryonic stem cells and showed that small RNA profiles can be used to distinguish between these cells types, HEK293FT and glioblastoma cells.

Faridani, O.R. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3701 (2016).

IMAGING

Optoacoustic imaging at multiple spatiotemporal scales

Optoacoustic imaging, also known as photoacoustic imaging, is gaining popularity because it does not have the same limitations as optical imaging when visualizing structures deep within tissues or even throughout mice. However, generating high-resolution 3D images of organs and whole animals with good temporal resolution is challenging. Deán-Ben *et al.* have begun to overcome these limitations with their method, spiral volumetric optoacoustic tomography (SVOT). In SVOT, optoacoustic image data are acquired along a spiral scanning trajectory using a spherical matrix ultrasound detection array, which enables real-time 3D imaging. The researchers demonstrated 3D tomography of whole mice in 5 minutes, and they were able to do fast imaging of mouse cardiac dynamics. This fast imaging enabled the team to clearly distinguish between heart perfusion dynamics and faster cardiac and respiratory motion.

Deán-Ben, X.L. *et al. Light Sci. Appl.* **5**, e16201 (2016).