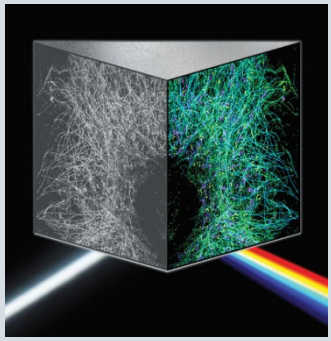
Highly multiplexed imaging

Methods for imaging multiple targets in a single cell are breaking the color barrier.

A major obstacle to visualizing biological structures in all their complexity is spectral overlap between fluorophores. This 'color barrier' practically limits most experiments to the examination of three or four targets. But what if we could see cells in many colors? Multiplexed imaging could make it possible to view structures in a more meaningful context and allow better analysis of how multicomponent complexes form and change in cells. It is poised to transform our understanding of biological processes.

Several strategies seek to address current issues with multiplexed imaging. Developers are designing better probes; these include probes that span the visible region and beyond for more color options, as well as probes such as intracellular lasers (*Nat. Photonics* **9**, 572–576, 2015; *Nano Lett.* **15**, 5647–5652, 2015) that have very narrow spectra and are therefore relatively easy to resolve from one another. These



Samuel Kenny, Zhengyang Zhang & Ke Xu

Imaging in many colors.

improvements could enable better multiplexed imaging with standard microscopes.

Researchers are also developing optical setups to detect overlapping fluorophores. For example, Ke Xu and colleagues reported spectrally resolved stochastic optical reconstruction microscopy (SR-STORM) in which both the position and the spectra of individual labeled molecules are measured for true-color super-resolution imaging (*Nat. Methods* **12**, 935–938, 2015). With such methods, even fluorophores with high spectral overlap can be readily discriminated, opening the door to imaging of numerous targets.

Methods for highly multiplexed immunofluorescence imaging are also emerging. In these methods, a subset of targets is labeled with antibodies and imaged. These probes are then rendered invisible by bleaching or stripping followed by additional rounds of the same labeling process against different targets. This allows highly multiplexed images to be built up over multiple rounds.

A related strategy has been used for highly multiplexed transcriptome imaging in which, over multiple rounds of imaging, individual transcripts are identified by a unique barcode (*Nat. Methods* **11**, 360–361, 2014; *Science* **348**, aaa6090, 2015). In the case of the MERFISH approach from the Zhuang lab, thousands of transcripts are imaged using only a single color.

Although these methods are powerful, improved methods are still needed for multiplexed imaging in live cells. Live-cell approaches have the same issues as fixed-cell approaches, but they also have other challenges, such as a smaller range of useful fluorescent dyes and probes, the need for rapid image acquisition, and sensitivity to light exposure. Methods to achieve massively multiplexed imaging will continue to be developed, enabling the study of biological processes as they occur in cells. **Rita Strack**

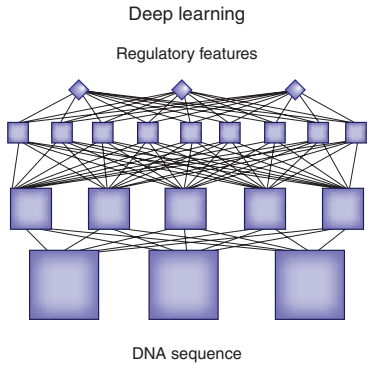
Deep learning

New computational tools learn complex motifs from large sequence data sets.

A powerful form of machine learning that enables computers to solve perceptual problems such as image and speech recognition is increasingly making an entry into the biological sciences. These deep-learning methods, such as deep artificial neural networks, use multiple processing layers to discover patterns and structure in very large data sets. Each layer learns a concept from the data that subsequent layers build on; the higher the level, the more abstract the concepts that are learned. Deep learning does not depend on prior data processing and automatically extracts features. To use a simple example, a deep neural network tasked with interpreting shapes would learn to recognize simple edges in the first layer and then add recognition of the more complex shapes composed of those edges in subsequent layers. There is no hard and fast rule for how many layers are needed to constitute deep learning, but most experts agree that more than two are required.

Recent examples show the power of deep learning to derive regulatory features in genomes from DNA sequence alone: DeepSEA (*Nat. Methods* **12**, 931–934, 2015) uses genomic sequence as input, trains on chromatin profiles from large consortia such as ENCODE and the Epigenomics Roadmap, and predicts the effect of single-nucleotide variants on regulatory regions such as DNase hypersensitive sites, transcription factor–binding sites and histone marks. Basset (bioRxiv, doi:10.1101/028399, 2015) uses similar deep neural networks to predict the effect of single-nucleotide polymorphisms on chromatin accessibility. DeepBind (*Nat. Biotechnol.* **33**, 831–838, 2015) finds protein-binding sites on RNA and DNA and predicts the effects of mutations.

Deep learning will be invaluable in the context of big data, as it extracts high-level information from very large volumes of data. As it gains traction in genome analysis, initial challenges such as overfitting due to rare dependencies in the training data



Computation that leads from sequence to functional annotation.

and high computational costs are being tackled. Researchers in academic settings as well as in startup companies such as Deep Genomics, launched July 22, 2015, by some of the authors of DeepBind, will increasingly apply deep learning to genome analysis and precision medicine. The goal is to predict the effect of genetic variants—both naturally occurring and introduced by genome editing—on a cell's regulatory landscape and how this in turn affects disease development. **Nicole Rusk**