unaffected. Remarkably, forced expression of S100b in the brown adipocytes of *Clstn3b*-knockout mice is sufficient to correct the deficits in sympathetic innervation and thermogenesis in these animals. The emerging picture is of an adipocyte-derived mechanism that mediates crosstalk between brown adipocytes and innervating sympathetic fibres (Fig. 1).

Zeng and colleagues' findings raise several questions. For example, how is calsyntenin 3β expression regulated in brown adipocytes? The authors found that one upstream regulator of this protein is the enzyme lysine-specific demethylase 1 (LSD1), which mediates the differentiation of brown and beige fat. Adipose-specific loss of LSD1 in mice causes downregulation of BAT-specific genes and aberrant induction of genes typically found in white adipose tissue[6].

The growth of sympathetic neurons mostly relies on a protein called nerve growth factor (NGF). NGF-deficient mice have pronounced disruption of sympathetic innervation of multiple peripheral tissues[7], but BAT innervation has not been specifically examined in this context. It remains to be seen whether NGF acts together with S100b to coordinate BAT innervation, or is dispensable, as in the innervation of the trachea[7]. Could other factors derived from peripheral tissue control the sympathetic innervation of specific targets? If so, they could be useful tools for manipulating such innervation and studying its role in individual tissues.

Sympathetic innervation controls the expression of genes involved in thermogenesis and the differentiation of brown and beige fat[4]. However, Zeng *et al*. found that loss of *Clstn3b* does not affect the expression of genes involved in adipocyte development, thermogenesis or mitochondrial function, including the gene that encodes UCP1. Only the expression of the *Dio2* gene, which encodes an enzyme that produces the active form of a thyroid hormone, is significantly altered by the loss or overexpression of *Clstn3b*. The Dio2 protein is abundant in BAT, and, as is seen in the *Clstn3b*-knockout mice, mice lacking *Dio2* have impaired thermogenesis and decreased cold tolerance, and are susceptible to diet-induced obesity[8]. Altered thyroid signalling might therefore contribute to BAT dysfunction in the absence of calsyntenin 3β.

The final issue arising from Zeng and colleagues' work is how calsyntenin 3β promotes the secretion of S100b. The synthesis of proteins destined for cellular secretion is initiated in the cytoplasm. The nascent proteins typically contain an amino-acid sequence known as a signal peptide, which directs them from the cytoplasm to the ER for the start of their secretory journey while they are still being synthesized. However, some secreted proteins lack signal peptides and are imported to the ER after they have been synthesized[9]. S100b lacks a peptide signal, and so the new findings raise the question of whether calsyntenin 3β functions as a general ER chaperone for the secretion of proteins that lack signal peptides, or is specific for S100b.

In humans, BAT had been thought to exist only in infants, until imaging studies revealed deposits of thermogenic brown fat in adults[10]. *Clstn3b* is expressed in human adipose tissue, and might be involved in the regulation of BAT innervation, as it is in mice. Zeng and colleagues' study might therefore inform therapeutic strategies to enhance sympathetic innervation, and thereby harness the thermogenic potential of BAT to combat obesity and its metabolic consequences. ■

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1. Cannon, B. & Nedergaard, J. *Physiol. Rev.* **84**, 277–359 (2004).
2. Zeng, X. *et al. Nature* **569**, 229–235 (2019).
3. Bartness, T. J., Vaughan, C. H. & Song, C. K. *Int. J. Obesity* **34**, S36–S42 (2010).
4. Wang, W. & Seale, P. *Nature Rev. Mol. Cell Biol.* **17**, 691–702 (2016).
5. Ludwin, S. K., Kosek, J. C. & Eng, L. F. *J. Comp. Neurol.* **165**, 197–207 (1976).
6. Duteil, D. *et al. Cell Rep.* **17**, 1008–1021 (2016).
7. Glebova, N. O. & Ginty, D. D. *J. Neurosci.* **24**, 743–751 (2004).
8. Hall, J. A. *et al. Endocrinology* **151**, 4573–4582 (2010).
9. Rabouille, C. *Trends Cell Biol.* **27**, 230–240 (2017).
10. Nedergaard, J., Bengtsson, T. & Cannon, B. *Am. J. Physiol. Endocrinol. Metab.* **293**, E444–E452 (2007).

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TRANSCRIPTION

Gene-expression maps fall into place

Knowing the gene-expression pattern of individual cells can unlock their identity. A refined method for generating cellular RNA profiles offers a way to obtain such data at a high level of spatial resolution in intact tissues.

SAMANTHA A. MORRIS

Monitoring messenger RNA in cells is a way to gather data that provides many biological insights. Improvements in the methods for detecting cellular mRNA are helping to provide more-detailed pictures of gene expression in the cells of a particular tissue. Writing in *Science*, Rodriques *et al.*[1] describe a new approach to assessing cellular RNA that they term Slide-seq. This versatile technique couples high-throughput RNA sequencing with a way to capture spatial information about the location of the analysed cells in tissues.

Scientists often try to tackle the complexity of a biological system by breaking it down to focus on cells, and cataloguing these individual units on the basis of their identity. Conventional microscopic imaging of cells in a tissue can provide much information, such as cell type and function, but imaging data of many cells can still lack a high level of detail about cellular features — the characteristics known as the cellular phenotype.

The development of techniques for rigorously analysing the molecular contents of individual cells offers an alternative way to capture thousands of cellular features and generate an unbiased picture of the cells in a given tissue. For example, single-cell RNA sequencing has been rapidly and widely adopted since its emergence ten years ago[2]. The use of microfluidic-based technologies in this approach has brought huge efficiency gains and cost reductions[3–5]. However, single-cell RNA sequencing requires tissue disruption and cell destruction, which causes loss of spatial information about cellular location that would be valuable for cell-type identification.

Efforts to retain spatial information are a current focus in the development of methods for single-cell analysis. For example, a method called multiplexed *in situ* hybridization, along with certain sequencing techniques, has enabled RNA to be monitored on a subcellular scale in intact tissues[6–8]. However, these approaches require substantial technical expertise, which has limited their widespread adoption.

Rodriques and colleagues' approach is conceptually rooted in an earlier[3] method for single-cell RNA analysis. In that technique, called Drop-seq, the tissue being analysed is disrupted to separate the cells, which are then loaded onto a microfluidic device. Individual cells are encapsulated in nanolitre-scale droplets together with a microbead coated with copies of a DNA sequence that provides a unique 'barcode' for the identification of material from each droplet (which is assumed to contain a single cell). The cells are broken open, which allows the cellular RNA to bind to the DNA barcodes. A step called reverse transcription generates DNA corresponding to the captured RNA sequences, and this DNA is tagged with the barcode DNA. DNA sequencing then enables

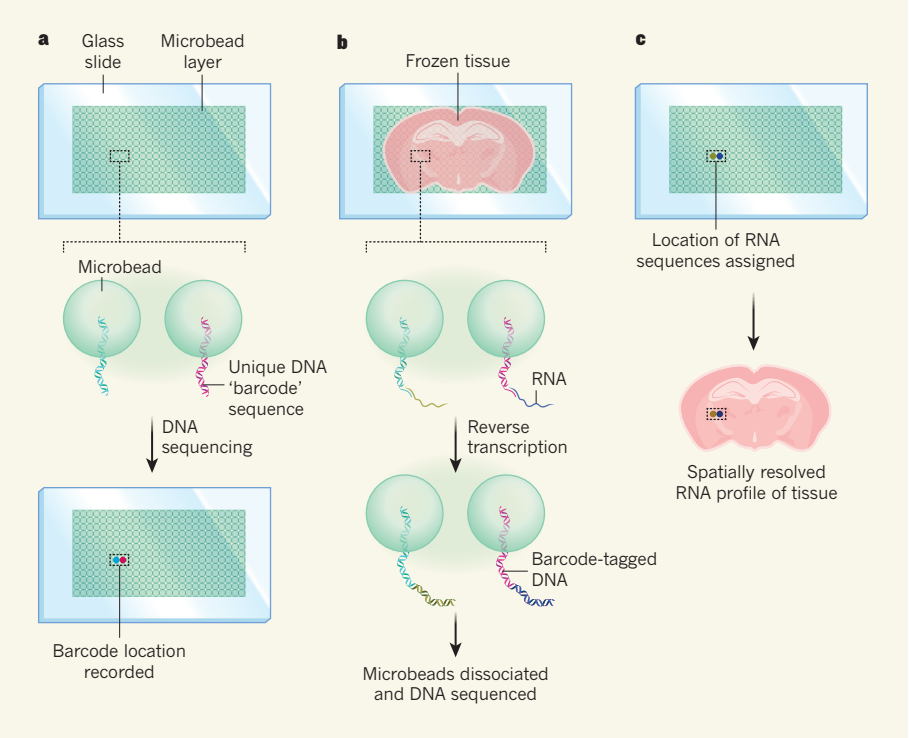

Figure 1 | Recording the spatial location of RNA sequences in tissues. Rodriques *et al.*[1] report a technique called Slide-seq, which enables RNA in frozen tissue samples to be observed in a way that provides spatial information about gene-expression patterns. **a**, The first step is the preparation of glass slides that contain a layer of microbeads. Each microbead has copies of a unique DNA sequence attached that provides an identification 'barcode' (only one barcode shown per bead). Sequencing these barcodes directly on the slide allowed the authors to record the slide position associated with each barcode. **b**, Rodriques and colleagues placed a frozen sample of tissue, such as a sample of mouse brain, on the slide. The cellular RNA bound to the underlying barcode DNA. An enzyme-mediated step called reverse transcription generated a DNA sequence corresponding to the RNA, and this DNA was attached to the barcode. The microbeads were dissociated from the slide and the DNA was sequenced. **c**, DNA-sequence analysis enabled the authors to generate a map of where specific RNA sequences were expressed in the tissue.

all of the RNA sequences associated with a particular barcode sequence to be deduced. Drop-seq provided a huge increase in the numbers of individual cells that could have their RNA sequences determined in a standard experiment.

With Slide-seq, Rodriques and colleagues developed an innovative twist on the Drop-seq approach by assembling the DNA-barcoded microbeads in a layer on a glass slide (Fig. 1). DNA sequencing of this material on the slide allowed the authors to determine the position and sequence of the barcodes corresponding to each microbead. A frozen tissue sample was then placed on the microbead layer, and RNA from the cells was captured by the underlying beads. As with Drop-seq, in subsequent DNA sequencing steps, the unique barcodes allowed each RNA transcript to be traced back to its bead of origin, and hence allowed assembly of a bead-specific transcriptional profile. The ability to assign each RNA transcript to a specific microbead location enabled the RNA profiling information to be presented at a spatial resolution on the scale of individual cells in a tissue sample. Rodriques *et al.* confirmed that Slide-seq worked effectively for different tissue types, including mouse brain, liver and kidney, and for specimens of the human brain region called the cerebellum.

Slide-seq might not capture the full profile of RNA in single cells. For example, the RNA molecules bound to a microbead are not necessarily representative of an entire cell if only part of the cell is captured by a microbead. Alternatively, if a microbead is positioned at the boundary between two cells, RNA from both cells might be recorded. Therefore, to infer the single-cell identity corresponding to each microbead profile, the authors turned to more-comprehensive single-cell transcriptional data collected in previously published single-cell RNA sequencing work. Using these data sets, the authors constructed a cell-type 'dictionary' to infer cell identity from the Slide-seq data. This strategy required the development of a computational approach to reconstruct each Slide-seq bead gene-expression profile, based on a weighted combination of the cell-type transcriptional signatures detected in a corresponding single-cell RNA-sequencing experiment.

Rodriques *et al.* conducted a range of experiments that showcased Slide-seq's power. They investigated the RNA profiles of cells in the hippocampal region of the mouse brain, using a strategy to capture the 3D structure of this region by analysing 66 sequential tissue sections. The authors generated RNA profiles of 1.5 million microbeads, demonstrating that Slide-seq can be scaled up as necessary. Their method identified gene-expression patterns that could be used to assign distinct cerebellar cell types together into spatially defined subpopulations in the mouse brain. Such expression of many genes is spatially dependent and is independent of cell type, but this valuable information is usually lost in conventional single-cell profiling approaches during the cell-dissociation step[9]. The authors also characterized cellular transcription dynamics in a mouse model of traumatic brain injury, revealing that an initial wave of cell proliferation after injury was followed by cellular differentiation.

One limitation of Slide-seq is that information about the size and shape of each cell and other key physical properties is not determined, unlike the case for RNA-profiling methods that use a microscopy technique called histology. A *Nature* paper[10] published in March offers a way to capture such cellular features while also tracking the subcellular localization of mRNA — this technique to image RNA transcripts in cells in tissues is called seqFISH+. Previous approaches similar to seqFISH+ were restricted by the optical-resolution limits of microscopes and the high density of mRNA transcripts in a cell. SeqFISH+ overcame these problems through a method that sequentially uses probes that provide an imaging palette of 60 colours as a tool with which to monitor individual mRNA molecules. This considerable palette breadth allows each mRNA to be localized as a dot in an individual cell using a confocal microscope, enabling the expression of about 10,000 genes to be visualized.

Together, Slide-seq and seqFISH+ represent valuable and complementary tools to aid major advances in efforts to retain spatial information during transcriptional analyses of cells. A key feature of these tools lies in their relative ease of use. Before the development of SeqFISH+, similar types of approach required a complex imaging system called super-resolution microscopy. Slide-seq is perhaps even more accessible, with experimental costs of just a few hundred US dollars and only about 40 hours of work required to prepare the materials needed for the sequencing step. It is an exciting time in this field. A commitment to create access to these technologies will facilitate their adoption and continued development, placing them at the heart of current efforts to construct high-resolution cellular atlases. ■

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1. Rodriques, S. G. *et al. Science* **363**, 1463–1467 (2019).
2. Tang, F. *et al. Nature Methods* **6**, 377–382 (2009).
3. Macosko, E. Z. *et al. Cell* **161**, 1202–1214 (2015).
4. Klein, A. M. *et al. Cell* **161**, 1187–1201 (2015).
5. Zheng, G. X. *et al. Nature Commun.* **8,** 14049 (2017).
6. Shah, S., Lubeck, E., Zhou, W. & Cai, L. *Neuron* **92**, 342–357 (2016).
7. Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S. & Zhuang, X. *Science* **348**, aaa6090 (2015).
8. Lee, J. H. *et al. Science* **343**, 1360–1363 (2014).
9. Zhu, Q., Shah, S., Dries, R., Cai, L. & Yuan, G. C. *Nature Biotechnol.* **36**, 1183–1190 (2018).
10. Eng, C.-H. L. *et al. Nature* **568**, 235–239 (2019).

NANOTECHNOLOGY

A role for optics in AI hardware

Experiments show how an all-optical version of an artificial neural network — a type of artificial-intelligence system — could potentially deliver better energy efficiency can conventional computing approaches. SEE ARTICLE P.208

GEOFFREY W. BURR

Optical fibres transmit data across the world in the form of light and are the backbone of modern telecommunications[1]. However, when such data need to be analysed, they get converted from light into electrons and are then processed using electronics. There was a time when optics was considered as the basis for a potential computing technology[2], but it became difficult for optical computing to compete with the rapid improvements made by its electronic counterpart. In the past few years, however, concern has been growing about the energy costs of computation. Therefore, optics is receiving attention again, both as a way to decrease energy requirements[3], and as a special-purpose hardware for accelerating artificial-intelligence algorithms such as deep neural networks (DNNs). On page 208, Feldmann *et al.*[4] report an intriguing advance towards all-optical implementations of such networks.

A DNN comprises many layers of artificial neurons and artificial synapses, which are connections between the neurons. The strengths of these connections are called weights and can be either positive, indicating neuronal excitation, or negative, implying inhibition. A DNN learns to perform tasks such as image recognition by varying its synaptic weights in a way that minimizes the difference between its actual output and the desired output.

Central processing units and other digital-based hardware accelerators[5] are typically used for DNN computations. A DNN can be trained using a known set of data, whereas an already trained DNN can be applied to unknown data in a task called inference. In either case, although the amount of computation is vast, the variety of operations is modest, because 'multiply–accumulate' operations dominate across the many synaptic weights and neuronal excitations.

DNNs are known to still work well when computational precision is low[5]. As a result, these networks represent an intriguing opportunity for unconventional computing techniques. For example, researchers are exploring DNN accelerators that are based on emerging non-volatile memory devices[6,7]. Such devices retain information even when their power source is switched off, and can offer improved speed and energy efficiency for DNNs through analog electronic computation.

Why not, therefore, also consider optics? Structures that direct light — whether they be an optical fibre for use in telecommunications or a waveguide patterned onto a photonic chip — can be packed with vast amounts of data. Inside such a waveguide, many wavelengths of light can propagate together, using a technique known as wavelength division multiplexing. Each wavelength can then be modulated (altered in such a way that it can carry information) at a rate that is limited by the available bandwidths associated with electronic-to-optical modulation and optical-to-electronic detection.

Structures called resonators enable individual wavelengths to be added to or removed from the waveguide, like wagons on a freight train. For example, micrometre-scale, ring-shaped (micro-ring) resonators can implement arrays of synaptic weights[8]. Such resonators can be modulated thermally[9], electro-optically[10,11] or, as in Feldmann and colleagues' work, through phase-change materials[12]. These materials can switch between an amorphous phase and a crystalline phase, which differ greatly in their ability to absorb light. Under ideal conditions, the resulting multiply–accumulate operations would require only a small amount of power.

Feldmann *et al.* present an all-optical neural network on a millimetre-scale photonic chip, in which there are no optical-to-electronic conversions within the network. Inputted data are electronically modulated onto different wavelengths for injection into the network, but after that has been performed, all the data stay on the chip. Both weight modulation and neuron integration are achieved using integrated phase-change materials; these are located on two types of micro-ring resonator, which have a synaptic or neuronal function.

Unmodulated light that is injected at the various operating wavelengths picks up the neuronal excitations that have accumulated in the phase-change material, and then passes them to the next layer of the network. Even without on-chip optical gain (a process in which a medium transfers energy to the light that is transmitted through it), this set-up could potentially be scaled up to larger networks. The authors demonstrate, on a small scale, both supervised and unsupervised learning — that is, training is achieved using labelled data, which is how DNNs learn, and using data without such labels, which is how humans tend to learn.

Because the weights are implemented by light absorption, negative weights require a large bias signal, which must not activate the phase-change material. An alternative approach[13] that can readily offer negative weights uses devices called Mach–Zehnder interferometers. In these devices, a single waveguide is split into two arms and then recombined; this causes the amount of transmitted light to depend on the difference in optical phase between the two paths. However, it might be challenging to combine this approach with wavelength division multiplexing, because the arms of each interferometer would need to introduce the appropriate phase difference for each wavelength.

Photonic DNNs still present substantial challenges. Their total power usage can be low in ideal situations, but thermo-optic power is frequently required to adjust and maintain the differences in optical phase in the arms of each Mach–Zehnder interferometer. Moreover, the total optical power that is injected into a system containing phase-change materials must be calibrated carefully, so that the materials respond to incoming signals exactly as intended. Although phase-change materials can also be used to adjust Mach–Zehnder phases, unavoidable cross-coupling between how strongly the materials absorb light and how much they slow it down poses a considerable complication.

Phase-change materials seem to be well suited for the non-volatile long-term storage of synaptic weights that are based on micro-ring resonators needing only infrequent adjustment. However, when used in the role of neuron, the speed of crystallization of such materials will limit the maximum rate at which neurons can be excited. Furthermore, the need to melt the materials to induce a full neuronal reset after every potential excitation event will rapidly consume the large, but finite,