Two-photon microscopy can image cells as deep as 1 mm. These cortical neurons were imaged at 200 µm.

of Colorado Anschutz Medical Campus in Aurora. He and his collaborators have eliminated the up-and-down motion required for focusing by using a liquid objective lens that is controlled by electric field. "When you throw oil on water, you form a lens," explains Restrepo. By making the lens very small, he and his colleagues have managed to make it very stable, so that it doesn't bobble about as an animal moves. And they can change the lens' shape and focal plane by altering the electrical field. Restrepo's team has used this lens in combination with a confocal microscope and a fibre-optic system to image brain slices[6], and now plan to attach the device to a mouse's head.

"We're literally still scratching the surface."

At University College London, neuroscientist Angus Silver found a way to accelerate the focus changes while imaging across multiple focal planes. He uses an acousto-optic lens that transmits megahertz sound waves through tellurium dioxide crystals to focus the laser beam. "The limitation to speed is the speed of sound across crystal, basically," Silver says. The technique still isn't ideal for quickly imaging every neuron in a volume, he says, but it can move from one region to the next in about 25 microseconds[7]. That makes it useful for viewing all of a sparse population, such as inhibitory interneurons in a volume of brain, he suggests.

Another solution to quickly sampling different depths is a modification of light-sheet microscopy, which typically involves moving multiple lenses to continually refocus a sheet of light. The technique can image one or two volumes per second, Hillman estimates. But by turning the sheet on an angle and using a single mirror to sweep it across the volume of interest, Hillman's group achieved a rate of 20 times per second. Hillman calls the technique swept confocally aligned planar excitation, or SCAPE, and her team has used it to visualize dozens of distinct firing patterns in the brains of awake mice[8]. The technology has been licensed to Leica Microsystems in Wetzlar, Germany.

Yuste's group offers yet another option. It uses a spatial light modulator, which splits the laser beam into many beamlets, each of which is aimed at a different part of the tissue. "Imagine a comb of light that's hitting the sample," Yuste explains. The microscope picks up any light that comes back, so it can capture multiple planes at once[9]. It can collect about ten sets of images per second, and the researchers are already speeding that up, Yuste says. Yuste has licensed the technology to Bruker in Billerica, Massachusetts, and Olympus in Tokyo, and is contemplating starting his own company.

ZOOMING IN, ZOOMING OUT

Most 2D and 3D techniques remain hampered by how the brain scatters light, but scientists have ways of circumventing that limitation, too. At Cornell University in Ithaca, New York, applied physicist Chris Xu and his colleagues reasoned that if two photons could push the imaging depth to a millimetre or so, then three should go even deeper. Indeed, Xu's three-photon imaging can reach two or three times further down than two-photon imaging can, he says, although the limits depend on the properties of the tissue being imaged. His group managed to use the technique to image the mouse hippocampus, without removing any of the cortex above[10].

Xu's team still can't penetrate all the way through the brain — "We're literally still scratching the surface," he acknowledges — but there's plenty of room for improvement, he says.

There's also room to develop live-brain imaging in other ways. A number of researchers, including Kleinfeld and Svoboda, have devised systems that combine the wide mesoscopic field of view with the single-cell resolution achieved by two-photon imaging, allowing them to zoom out on much of the brain or zoom in, Google Earth-style, on individual neurons[11,12]. Kleinfeld's field-of-view covers an 8×10 millimetre section of cortex; Svoboda's group can manage a cylinder of brain about 5 millimetres in diameter and 1 millimetre deep, and that's about 25 times the typical field-of-view in two-photon microscopy, he says. Svoboda has now trained several labs to build their own versions of his microscope, and licensed the technology to Thorlabs in Newton, New Jersey.

Ultimately, these diverse technologies could realize Yuste's dream for neuroscience: to "crack the code" that links neural firing patterns with behaviour and sensation. The technology can't yet be used to look at and interpret the activity in a mouse's visual cortex, for instance, but it has certainly added plenty of pixels to the screen. ■

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1. Malvache, A., Reichinnek, S., Villette, V., Haimerl, C. & Cossart, R. *Science* **353,** 1280–1283 (2016).
2. White, B. R. *et al. PLoS ONE* **6,** e16322 (2011).
3. Bauer, A. Q. *et al. NeuroImage* **99,** 388–401 (2014).
4. Bero, A. W. *et al. J. Neurosci.* **32,** 4334-4340 (2012).
5. Zou, P. *et al. Nature Commun.* **5,** 4625 (2014).
6. Ozbay, B. N. *et al. Opt. Lett.* **40,** 2553–2556 (2015).
7. Naga Srinivas Nadella, K. M. *et al. Nature Methods* http://dx.doi.org/10.1038/nmeth.4033 (2016).
8. Bouchard, M. B. *et al. Nature Photon.* **9,** 113–119 (2015).
9. Yang, W. *et al. Neuron* **89,** 269–284 (2016).
10. Horton, N. G. *et al. Nature Photon.* **7,** 205–209 (2013).
11. Sofroniew, N. J., Flickinger, D., King, J. & Svoboda, K. *eLife* **5,** e14472 (2016).
12. Tsai, P. S. *et al. Opt. Express* **23,** 13833–13847 (2015).

CORRECTION

The Technology Feature 'The dark side of the human genome' (*Nature* **538,** 275–277; 2016) implied that Ran Elkon was solely responsible for performing the first screen using the advanced editing system. In fact, he was part of the team headed by Reuven Agami.