team has achieved still further improvements in axial resolution with an iteration of STORM that resolves all three dimensions at 10 nanometres[8].

Imaging success depends on more than just the instruments: the sample itself is also an important consideration. Tissue specimens are especially hard to image because they are dense and tend to scatter photons, generating blurry images and high levels of background fluorescence. As a result, image quality is best near the sample's surface and worsens as the microscope probes deeper into thick samples. It may be possible to overcome this hurdle by using chemical 'clearing' techniques that render tissues transparent.

For now, most researchers find that the simplest solution is to embed fixed samples in plastic, and then sequentially image thin slices shaved off the top. "We're trying to understand the relationship of one synapse with different postsynaptic partners, which requires us to look at thousands of synapses in tissue at high-resolution in parallel," says Bernardo Sabatini, a neurobiologist at Harvard Medical School. "I think that in the short term, this approach plus super-resolution will give you that data quickly."

PICTURE PERFECT

Even a perfectly executed super-resolution study generally needs some sort of computational processing to produce a high-quality image. For scientists who prefer the simplicity of positioning a sample under a microscope and having the image instantly appear on a computer screen, STED might be best because it generally does not require image processing.

Some scientists use deconvolution tools to sharpen images and eliminate blur, but Hell avoids this whenever possible. "Raw data may not look as fancy, but it's honest, and you know what it means," he says. "For most other techniques, software processing is mandatory." And Davis says of SIM, "You're creating a mathematical model of what the cell looks like based on the fluorescence data. You're not literally seeing it."

Raff notes that many of his early experiences with SIM entailed recognizing that pretty pictures can be deceiving because image-processing algorithms can create artefacts that look every bit as real as the cellular structure of interest. "But if you have people who know what to look for, they can examine the image and tell if something is dodgy," he says.

For PALM and STORM, image-building is like a game of 'join the dots'. The higher the density of the labels, the easier it is for the software to connect those dots, leading to better images. But high density can also cause confusion, by generating overlapping signals that look like single dots — so clever use of powerful image-processing algorithms is essential to make sense of the data.

Given that most super-resolution techniques can be incorporated into existing microscopes, many researchers will probably try their hand at super-resolution imaging in the near future. "In my view, it doesn't make sense for a facility that routinely uses confocal microscopy not to have STED attached to it," says Hell. "You can just stop the STED beam and still have a confocal system."

Those with experience in nanoscopy are helping to train others. Zhuang's team at Harvard University, for example, offers routine STORM workshops. "We go from sample preparation to analysing images with our software," she says. "It's always oversubscribed."

That said, most biologists are still best served by using these instruments in core facilities that provide access to specialists who are familiar with several methods. "As biologists, we're still far away from understanding the physics — and some of us never will," says Raff. "Your best bet is to try multiple different techniques out on your sample in an environment where there are people around who understand it." ■

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A good way to dye

Microscopist Stefan Hell at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, thinks that all super-resolution methods boil down to one crucial element: "The dye is essential," he says. The ideal fluorophore has an extremely bright 'on' state and very dark 'off' state, and the capacity to switch between the two both rapidly and repeatedly.

For live-cell imaging, many researchers prefer to work with genetically encoded fluorescent proteins. Stimulated emission depletion (STED) and structured illumination microscopy (SIM) are highly compatible with standard fluorophores such as green fluorescent protein (GFP). Stochastic optical reconstruction microscopy (STORM) and photoactivation localization microscopy (PALM) need photoswitchable dyes; proteins such as Dendra2 or EosFP, which undergo a laser-induced colour transition, are popular choices.

But fluorescent proteins generally compromise resolution. "They're just too dim," says neurophysiologist Silvio Rizzoli at the Gottingen Graduate School for Neuroscience, Biophysics and Molecular Biosciences in Germany. In STED, "you're taking the laser power and genetic overexpression to the maximum to get a signal".

Organic dyes are a brighter alternative. They tend to be more durable under prolonged illumination. However, they must be linked to another molecule to achieve targeted labelling, and many fluorescent dyes cannot penetrate living cells. For this reason, many researchers still focus on fixed samples. "We'd rather go for the extreme in resolution, and we try to squeeze every single photon out so that we can localize things very accurately," says cell biologist Helge Ewers at the Free University of Berlin. A handful of high-performance dyes can be used with live cells, such as the silicon–rhodamine dyes from the Swiss bioimaging company SpiroChrome, which generate bright-red fluorescence once bound to cytoskeletal proteins.

Things get tricky when one aims to image many targets simultaneously using multicoloured labelling: because each fluorophore responds to a distinct 'on' and 'off' wavelength, researchers may run out of bandwidth to achieve specific detection of more than two or three tags. In principle, probe-based methods can accommodate more labels than STED, but they are also more finicky in terms of experimental conditions. "People often come to us with a combination they want to use, but the dyes have exact opposite needs in terms of buffers," says Christine Labno, technical director of the University of Chicago's Light Microscopy Core Facility in Illinois. Sequential-labelling strategies may offer a more efficient option for conducting larger-scale protein-mapping experiments. For example, a technique known as DNA-PAINT uses DNA tags to selectively conjugate a single dye to different antibodies, enabling stepwise labelling of ten or more protein targets in one super-resolution image. M.E.

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CORRECTION

The Technology Feature 'The cell menagerie: human immune profiling' (*Nature* **525,** 409–411; 2015) misstated the location and research focus of Hedda Wardemann. She is at the German Cancer Research Center in Heidelberg and focuses on single-cell sequencing.

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