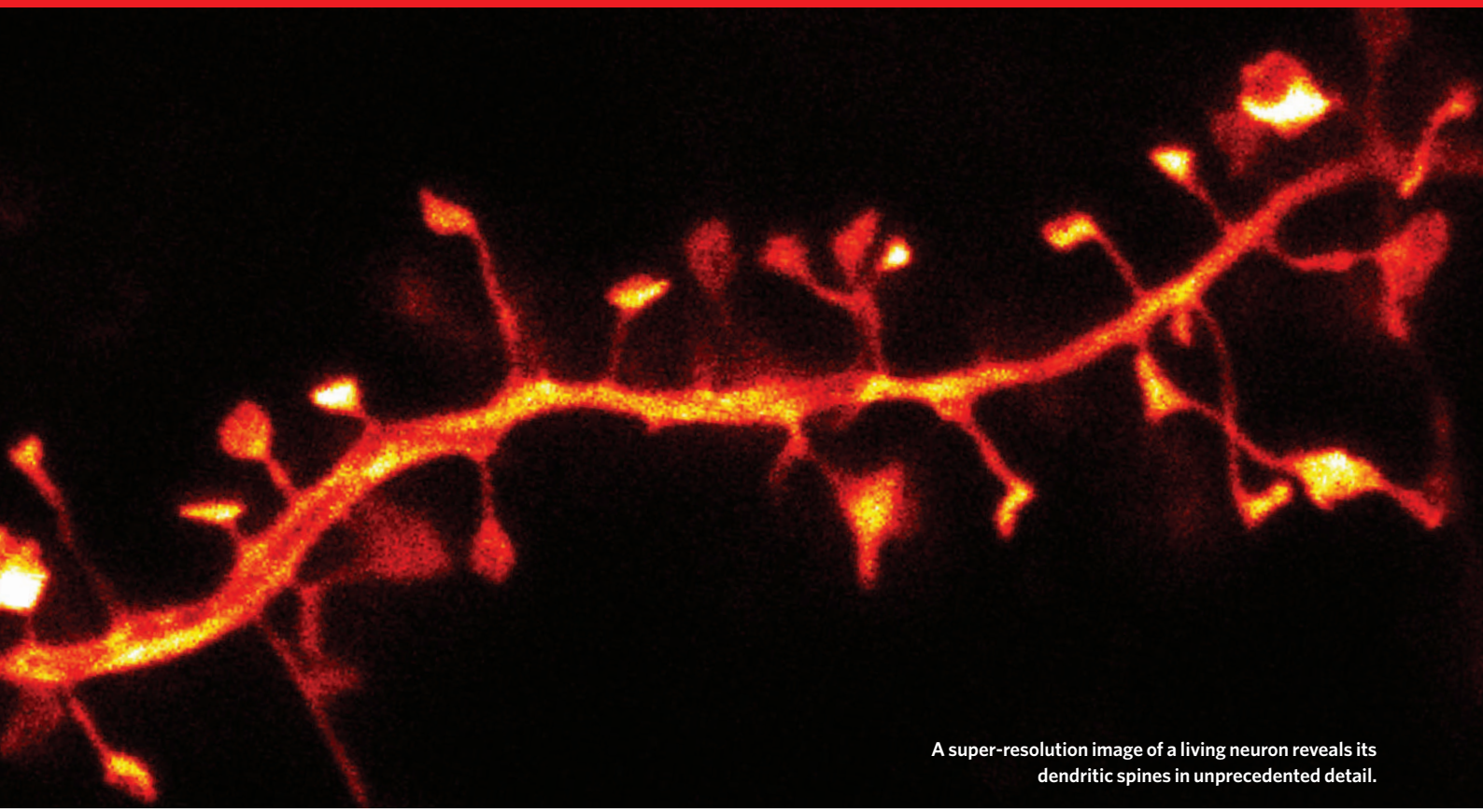


A super-resolution image of a living neuron reveals its dendritic spines in unprecedented detail.

K. WILLIG, V. NÄGERL, N. URBAN, T. BONHOEFFER, S. W. HELL /MAX PLANCK INST. BIOPHYS. CHEM.

THE GLORIOUS RESOLUTION

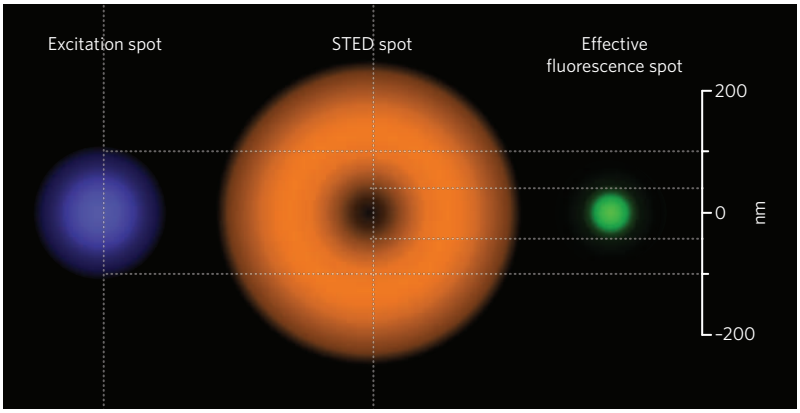
"It's childish, but it still gives me great pleasure to see high-res pictures everyone told me would be impossible," says Stefan Hell of the razor-sharp silhouettes of mouse neurons on his screen.

Hell can't resist a small gloat. He frequently refers to his bitter struggle for recognition — the many years in the 1990s trying to reinvent microscopy as nanoscopy, able to reveal structures an order of magnitude smaller than light microscopy. 'You can't argue with the law of physics', was the no-can-do attitude of most microscopists at the time. They were referring back to 1873, when German physicist Ernst Abbe declared that diffraction inevitably limits the resolution of microscopy to around half the wavelength of light.

Hell didn't argue with the laws of physics — he found a way around them. Hell's innovation was to think about how he might exploit the properties of the fluorescent dyes, or fluorophores, that are widely used to label molecules for microscopy in biological samples. Fluorophores can exist in three states: ground, excited and dark. The confocal microscope, a standard machine in cell biology, works by rapidly scanning a sample with a focused beam of light and capturing the photons emitted when fluorophores are activated by the beam from ground to excited state. In practice, the 'diffraction barrier' means that two fluorescent spots less than around 200–300 nanometres apart appear as one blurred blob.

It dawned on Hell that he could use another beam of light, of slightly longer wavelength, to switch the fluorophores on the edge of the blurry spot back to their ground state. So he added a second beam to his confocal microscope. This beam is fashioned like a tube of light with a hollow centre (see graphic). It hugs the excitation beam tightly, depleting all of the fluorescence in a sample except that at the very centre of each spot. This is the ingenious principle of stimulated emission depletion (STED) microscopy — and it throws objects measuring 20 nanometres into fine relief.

Hell dispelled much scepticism about his technique with a 2000 paper[1] in *Proceedings of the National Academy of Sciences* — "previously rejected by both *Nature* and *Science*," he observes ruefully — which showed that his STED method could generate nanoscale fluorescent images. The objects themselves (beads, yeast and bacteria) were not exciting, but the proof of principle was. Two years later he was appointed a director at the Max



Superimposing a doughnut-shaped STED beam on the blue excitation beam shrinks the fluorescent area in a sample (green) to tens of nanometres, below the diffraction limit.

Planck Institute for Biophysical Chemistry in Göttingen, Germany, with all the research money and facilities he could handle. "There is still refinement needed — we are working on being able to do the same things at lower light levels and with a larger field of view," says Hell. "But the basic principle has been cracked."

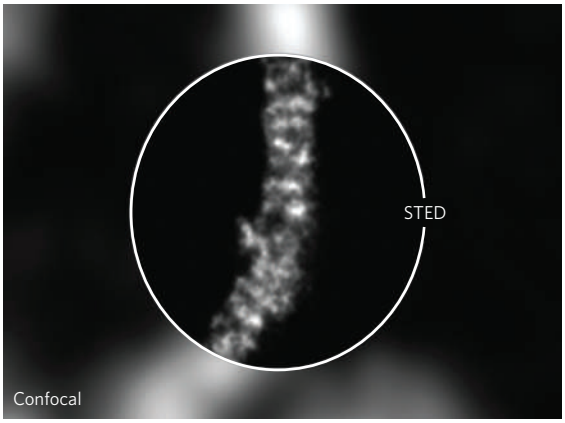
Hell now finds himself with stiff competition as other labs race to develop, improve and commercialize techniques for 'super-resolution' microscopy. US scientists have developed methods known as stochastic optical reconstruction microscopy (STORM) and photoactivated localization microscopy (PALM), which use computation to work out the location of individual fluorophores and compile them into high-resolution images. And in structured illumination microscopy (SIM), biological samples are illuminated with stripes of light rather than a focused beam, and the interference patterns of the stripes are analysed and used to reconstruct images on nanometre scales.

Biologists can't team up with the new nanoscopy labs fast enough. "It is coming at a really exciting moment in biology," says Jeffrey Lichtman, a cell biologist at Harvard University, "and particularly in neuroscience, because neural connections are — tantalizingly — just below the diffraction limit." In his energetic bid to map all the neural connections in the brain, Lichtman says he is collaborating with all the main super-resolution microscopy labs.

STED microscopy has already revealed cell biological details that had been lost in the fluorescent fog. A video accompanying Hell's 2008 *Science* paper[2] shows 40-nanometre-diameter vesicles moving within a nerve cell. Nothing like this had ever been seen before. The speed with which the vesicles zap around, like fleas on a blanket, is almost unseemly. It is one thing to appreciate the theory and mathematics of cell dynamics, but it is quite another to finally be able to see it in action.

Kai Simons, a cell biologist at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, has seen his own 'lipid raft' theory gain a slug of support from the Hell lab[3]. Simons's originally controversial theory, proposed in 1988, held that certain lipids and proteins in a cell membrane form temporary clumps to, for example, facilitate the transmission of signals across the membrane[4]. But the proposed rafts would have been as small as 30 nanometres in diameter — invisible until STED microscopy came along. Hell's group showed certain proteins and lipids being transiently trapped in the complexes, exactly according to the theory. "It has given us real biological insight — you can't achieve a dynamic view at this scale any other way," says Simons.

The technology will be crucial for a large number of biological questions."
— Tobias Bonhoeffer



STED reveals the folds of the mitochondrial inner membrane, previously visible only with the electron microscope.

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Although impressed by the technical advance, some researchers say that super-resolution microscopy has yet to prove itself. "I think these techniques owe us a real scientific breakthrough in solving a question that couldn't be solved any other way," says Winfried Denk, a microscopy pioneer based in at the Max Planck Institute for Medical Research in Heidelberg, Germany.

Others have no doubt that it will. For Tobias Bonhoeffer, a director of the Max Planck Institute of Neurobiology in Martinsried, Germany, STED microscopy is "a transforming technology which will be crucial for a large number of biological questions". In collaboration with Hell, he has published pictures of the tiny dendritic spines on neurons, which are central to processes such as information storage in the brain, learning and memory[5]. "Spines are around a micrometre across — you can see them with light microscopy, but not the details," he says. "With this increase in resolution, we can even see the fine filopodia reaching out to make connections." ■

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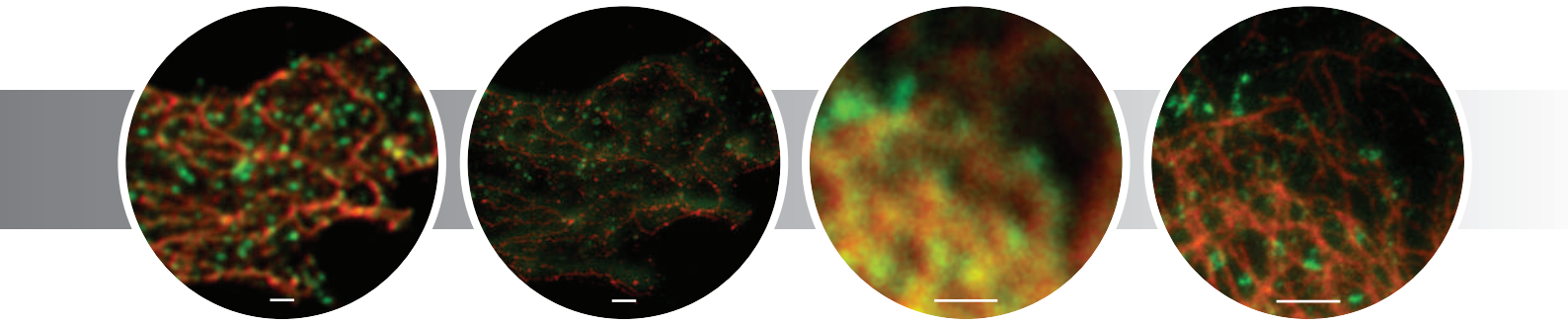

Side-by-side comparisons of human glioblastoma using a confocal microscope (first and third from left) and STED (second and fourth). Scale bars all 1 micrometre.

Alison Abbott

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See also page 629 and online at http://tinyurl.com/microspecial.