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Blown-up brains reveal nanoscale details

Material used in diaper absorbant can make brain tissue bigger and enable ordinary microscopes to resolve features down to 60 nanometres.

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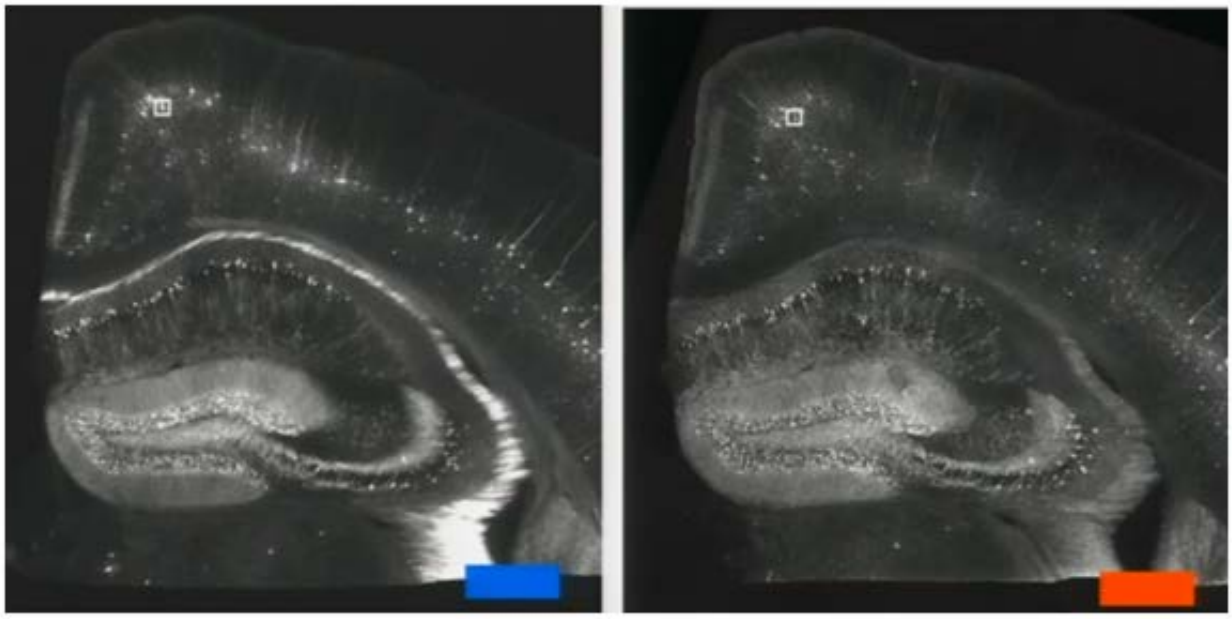

Boyden, E., Chen, F. & Tillberg, P. / MIT / Courtesy of National Institutes of Health

A slice of a mouse brain (left) was expanded by nearly five-fold in each dimension by adding a water-soaking salt. The result — shown at smaller magnification (right) for comparison — has its anatomical structures are essentially unchanged.

Microscopes make living cells and tissues appear bigger. But what if we could actually make the things bigger?

It might sound like the fantasy of a scientist who has read Alice's Adventures in Wonderland too many times, but the concept is the basis for a new method that could enable biologists to image an entire brain in exquisite molecular detail using an ordinary microscope, and to resolve features that would normally be beyond the limits of optics.

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The technique, called expansion microscopy, involves physically inflating biological tissues using a material more commonly found in baby nappies (diapers). Edward Boyden, a neuroengineer at the Massachusetts Institute of Technology (MIT) in Cambridge, discussed the technique, which he developed with his MIT colleagues Fei Chen and Paul Tillberg, at a conference last month.

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Prizewinning roots

Expansion microscopy is a twist on super-resolution microscopy, which earned three scientists the 2014 Nobel Prize in Chemistry. Both techniques attempt to circumvent a limitation posed by the laws of physics. In 1873, German physicist Ernst Abbe deduced that conventional optical microscopes cannot distinguish objects that are closer together than about 200 nanometres — roughly half the shortest wavelength of visible light. Anything closer than this 'diffraction limit' appears as a blur.

Super-resolution microscope methods overcome Abbe's limit by manipulating fluorescent molecules tethered to proteins, to better locate the source of the light the molecules emit. These methods can now discern objects that are as close together as about 20nm. But the finicky techniques require expensive, specialized equipment, and they struggle with thick structures, such as sections of brain or tumours.

Boyden and many other neuroscientists would like to glean molecular details such as the location of proteins at neural synapses — the junctions at which two neurons communicate — within a group of neurons or even across an entire brain.

"What we've been trying to do is figure out if we can make everything bigger," Boyden told the meeting at the US National Institutes of Health (NIH) in Bethesda, Maryland. To manage this, his team used a chemical called acrylate that has two useful properties: it can form a dense mesh that holds proteins in place, and it swells in the presence of water. Acrylate, a type of salt also known as waterlock, is the substance that gives nappies their sponginess. When inflated, Boyden's tissues grow about 4.5 times in each dimension.

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Just add water

Before swelling, the tissue is treated with a chemical cocktail that makes it transparent, and then with the fluorescent molecules that anchor specific proteins to the acrylate, which is then infused into tissue. Just as with nappies, adding water causes the acrylate polymer to swell. After stretching, the fluorescent-tagged molecules move further away from each other; proteins that were previously too close to distinguish with a visible-light microscope come into crisp focus. In his NIH presentation, Boyden suggested that the technique can resolve molecules that had been as close as 60nm before expansion.

Crucially, the process largely maintains the relative orientation and interconnection of proteins and keeps other cellular structures intact: it distorts the relative position of proteins by 1–4%, Boyden's team calculated.

Expansion microscopy stacks up well against other super-resolution techniques, according to Boyden. In one experiment with inflated mouse brain tissue, the researchers gauged the distance between two proteins that sit on opposite ends of neural synapses. Their measurement was nearly identical to one taken with a super-resolution technique[1].

But expansion microscopy, Boyden said, may do a better job of imaging complex tissue in three dimensions. At the meeting, he showed an image of a half-millimetre slab of the mouse brain's hippocampus, at a scale that revealed connections between neighbouring neurons. Zooming in on the same image even revealed details of minute synapse structures, called boutons, where neurotransmitters are released. Boyden's team has also worked on the brains of fruit flies and zebrafish, while a collaborating group is applying expansion microscopy to human brains.

Pushing boundaries

Viviana Gradinaru, a neuroscientist at the California Institute of Technology in Pasadena, says that Boyden's technique is another example of how scientists are bypassing hardware limitations by modifying biological tissue. In 2013, a team led by Gradinaru together with Karl Deisseroth of Stanford University in California reported a method that strips away fats and other molecules to make intact brain tissue transparent, allowing thick sections to be imaged with a light microscope[2] (see 'See-through brains clarify connections'). Last year, Gradinaru's team applied the technique to other organs and an entire mouse[3]. "This seems a wonderful story," she says of Boyden's approach.

"This is certainly highly ingenious, but how much practical use it will be is less clear," notes Guy Cox, a microscopy specialist at the University of Sydney, Australia. "If this is to be any serious use, I suspect it will be in collaboration with existing super-resolution techniques, on small macromolecular complexes, to push the boundaries a bit further, rather than looking at whole cells."

Stefan Hell, a director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, who shared last year's chemistry Nobel, says that the technique is interesting and worth pursuing. Scientists working at the University of Rostock in Germany proposed a similar idea in the early 1990s, Hell notes. "It seems that Boyden et al. found a solution that really works."

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References

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