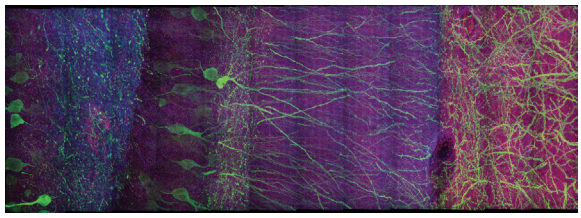
IMAGING

Bigger is better for super-resolution

Expansion microscopy uses enlarged samples for high-resolution imaging with conventional microscopes.



ExM image of a portion of a mouse hippocampus showing neurons in green, synapses in blue and Homer1 in red. Image reproduced from Chen *et al.*, AAAS.

Super-resolution microscopy has had a large impact on biological research, but it still has limitations in some applications. Edward Boyden and two of his graduate students, Fei Chen and Paul Tillberg, at the Media Lab and McGovern Institute at the Massachusetts Institute of Technology (MIT), were interested in mapping brain circuits. However, they found that imaging large three-dimensional (3D) biological specimens with nanoscale precision was very difficult using electron microscopy (EM) and conventional super-resolution imaging. "With EM you have to section into thin sections, and with optical microscopy many of the technologies either require very expensive equipment or they run very slowly," says Boyden. He adds that, with EM, "you cannot see the molecular information." For these reasons, Boyden and his team sought a new approach to enhance image resolution.

Their new method, called expansion microscopy (ExM), involves synthesizing a 'swellable' polymer network within a biological specimen, which enlarges the sample by roughly 4.5-fold. The enlarged sample can then be imaged using conventional microscopy with diffraction-limited resolution. After imaging, the image size is divided by the fold increase in sample size to effectively achieve super-resolution imaging with a spatial resolution of ~70 nanometers.

The idea for ExM started as "sort of fantasy," says Boyden, who was inspired by the work of the late Toyoichi Tanaka. Tanaka was a professor of physics at MIT who studied responsive polymers, or 'smart gels', which change their shape and size under different conditions. "[We] started playing with some of the varying molecules that [Tanaka] was playing with in the 1970s and 80s and found that it kind of worked," Boyden recalls. "If you synthesize these swellable polymers inside a tissue, then you can add water and swell the tissue."

However, despite the promising initial experiments, the researchers had to overcome several technical hurdles while developing ExM. "We could expand the tissue, but it would often crack because the natural molecules resist expansion," says Boyden. To refine their strategy, they developed a new labeling approach that allowed specific proteins to be labeled and the fluorescent probe to be anchored to the polymer. Then, when water was added and the polymer expanded, the fluorescent probe was taken along with it. This labeling strategy allowed the researchers to digest structural components of the samples with proteases, facilitating smooth expansion of the specimens.

Boyden notes that for ExM to be useful as a biological imaging technique, it has to be homogeneous and predictable. His team numerically validated the distortion associated with swelling by comparing pre- and post-expansion samples by imaging microtubules and clathrin-coated pits in mammalian cells grown in culture both pre- and post-expansion using confocal microscopy. This showed that distortion was very low.

Next, they compared their ExM results to super-resolution structured illumination microscopy (SR-SIM) data. In side-by-side comparisons of the same samples imaged with SR-SIM (pre-expansion) and ExM, they saw that ExM achieved better resolution. For microtubules, ExM allowed individual microtubules to be distinguished that were not resolvable in the SR-SIM images.

The team then tested whether ExM could be used to image fixed brain tissue from mice. They found that slices could be expanded to four times their original size. In this case, ExM imaging enabled the authors to visualize nanoscale features of the brain on length scales relevant to the study of neural circuits, moving them closer to the goal that set this work in motion.

According to Boyden, ExM has several advantages over conventional super-resolution imaging approaches. One is that it allows for imaging of large 3D objects, whereas conventional methods are often limited to a very small region of a cell and limited depth. ExM also does not require special probes, which are needed for point-localization super-resolution microscopy methods. This especially facilitates multicolor imaging, which is straightforward for ExM. Finally, a unique feature of ExM is that, as a side effect, the sample becomes transparent as it expands and is filled with water. This transparency could allow for rapid super-resolution light-sheet microscopy of expanded samples.

The future of this method may go beyond imaging. Boyden notes that expanded samples may take some assays, such as DNA sequencing, RNA sequencing and proteomic analyses, out of test tubes and into intact tissues by creating more room around the biomolecules and controlling buffer conditions. "If you can do *in situ*, 3D, nanoscale biomolecular identification and localization, this could really be useful for looking at the parts of the cell and how they're configured," says Boyden. "And you can really study how biomolecules are arranged and which biomolecules are in certain cells."

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RESEARCH PAPERS
Chen, F. *et al.* Expansion microscopy. *Science* **347**, 543–548 (2015).