GENE EXPRESSION

Expanding views of the transcriptome

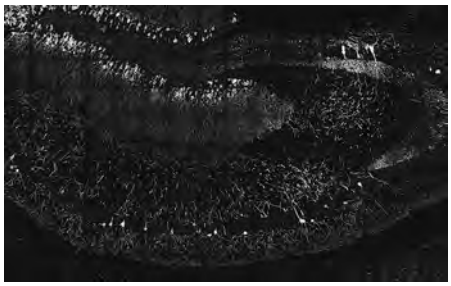
In situ long-read sequencing combined with expansion microscopy enables precise views of transcriptomes in intact biological systems.

Expansion microscopy (ExM) is a versatile method that has gathered a large user base since its initial development in the lab of Edward Boyden at the Massachusetts Institute of Technology (MIT) and publication in 2015. In ExM, samples such as cells and tissues are embedded with a swellable polymer that, along with other chemical steps, allows them to be swollen from four- to twenty-fold their initial size. Once expanded, the samples are largely optically clear and can be imaged with commonly available microscopes to achieve super-resolution. For example, samples that are swollen to four times their normal size and imaged with confocal microscopes can produce images with ~60 nm spatial resolution.

Since its inception, numerous extensions to ExM have shown the benefits of the technique for imaging a range of biomolecules, such as proteins, RNA, DNA and lipids, in diverse and challenging sample types, including intact *C. elegans* and formaldehyde-fixed and paraffin-embedded clinical samples. And it has become clear that this combination of sample de-crowding, optical clearing and compatibility with standard microscopes enables new investigations of biological structures.

Boyden's group further extended this technology to develop expansion sequencing (ExSeq) for in situ sequencing of expanded samples. This collaborative effort was co-driven by Adam Marblestone of MIT and the lab of George Church at Harvard University. Boyden was inspired in part by biological questions surrounding gene expression, especially in neurons. "Neurons have one nucleus but thousands of synapses. It makes sense that gene expression is regulated throughout neurons with great spatial precision," he notes. "Multiplexed gene expression mapping is powerful and was recently declared 'Method of the Year' by *Nature Methods*, but it's hard to do it with very high spatial precision. With expansion sequencing, we can map out expressed genes in tiny cellular compartments such as synapses, or in small volumes containing touching cancer cells and immune cells."

ExSeq involves first expanding and then chemically stabilizing the swollen specimen. Then sequencing chemistry is carried out on the sample to reveal both the position of an RNA and its identity at the nanoscale. As Boyden explains, "When you sequence a nucleic acid, you essentially copy its genetic sequence, using fluorescent nucleotides, so that each expressed gene will blink out its genetic sequence over time. We image each nucleic acid as each nucleotide is thus added, identifying the gene expressed at each location over many cycles of imaging."



ExSeq of genes in the mouse hippocampus. Credit: All authors of S. Alon et al. *Science* **371**, eaax2656 (2021), AAAS

ExSeq comes in two flavors, untargeted and targeted. In the untargeted version, expansion is combined with FISSEQ, an approach developed in the Church lab, and transcripts are sequenced at random. This allows transcriptome-wide exploration of even rare localized RNAs. The targeted version detects a smaller, predefined set of genes and is useful for mapping cell types and states along with the spatial relationship of specific transcripts in situ. For targeted ExSeq, the researchers employed oligonucleotide barcoded 'padlock' probes that hybridize to transcripts. These transcripts were amplified and then identified by in situ sequencing of the barcodes.

After demonstrating that ExSeq works on both cultured hippocampal neurons and mouse hippocampal slices, the researchers used both untargeted and targeted ExSeq to study the spatial regulation of gene expression. Using the untargeted approach, the team was able to study the positions of expressed genes in different areas of hippocampal neurons. The untargeted nature also allowed them to explore splice variants of transcripts and their positions.

The team gave several demonstrations of the targeted approach as well. In the first, they showed that they could identify neuron types of mouse primary visual cortex with high accuracy compared to that of other approaches. They then showed that they could characterize nanoscale transcriptomic compartments that are implicated in synaptic plasticity and learning in dendrites and spines of mouse hippocampal neurons. "It was interesting to compare the dendritic profiles of expressed genes across different cell types and to see common patterns emerge, which may suggest interesting principles of how gene expression is spatially regulated," notes Boyden of these results. They ended by demonstrating that targeted ExSeq can be used to map cell states as well as cell types in a metastatic breast cancer sample.

Getting ExSeq to work was not as simple as adding sequencing onto established ExM protocols. Boyden recalls, "One difficulty was that the charged nature of the expansion polymer, key to the expansion process, blocked the enzymatic reactions required for sequencing. We had to figure out how to passivate, or cancel out, the charge, while still keeping the sample in the expanded state. This was achieved by forming a stabilizing hydrogel throughout the specimen, to keep it expanded, and then cancelling out the charge by chemically treating the charged groups along the polymer." Another challenge was handling the data. "We easily could acquire terabytes of data from a single specimen. We had to work hard on the algorithmic, software and even hardware angle to efficiently analyze ExSeq data," says Boyden.

Although much has been achieved with ExM, Boyden still sees much room for growth with the technologies. "If we could map many kinds of biomolecules with single-molecule precision, that could be even more transformational."

Rita Strack

Published online: 7 April 2021
https://doi.org/10.1038/s41592-021-01119-1

Research paper
Alon, S. et al. Expansion sequencing: spatially precise in situ transcriptomics in intact biological systems. *Science* **371**, eaax2656 (2021).