New imaging technique can capture entire plant tissues in 3D

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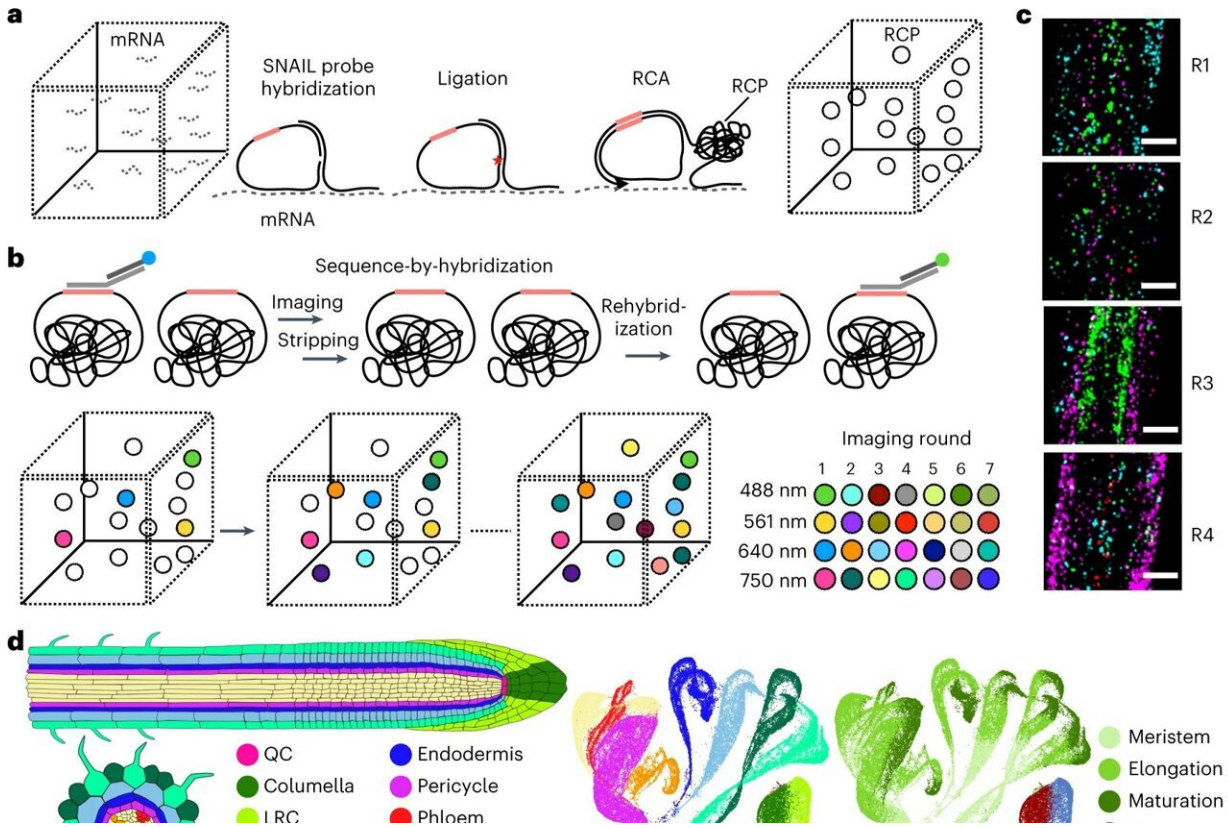

Whole-mount spatial mapping of root tip cell-type marker genes with PHYTOMap. **a**, In fixed whole-mount tissue, target mRNA molecules are hybridized by pairs of DNA probes (SNAIL probes) that harbor mRNA species-specific barcode sequences (pink bars). Barcode-containing DNA probes are circularized by ligation (red star) and amplified in situ by RCA. During amplification, amine-modified nucleotides are incorporated into the DNA amplicons (RCPs) and stably cross-linked with the cellular protein matrix using a non-reversible amine cross-linker. Amplified DNA barcodes are detected by

SBH chemistry through multiple rounds of imaging. **b**, SBH chemistry. Before each imaging round, four types of bridge probes are hybridized to a set of four DNA barcodes. Each bridge probe is then targeted by one of four fluorescent probes to be imaged. After imaging, bridge probes and fluorescent probes are stripped away, keeping RCPs in place. These steps are repeated until all the DNA barcodes are read. **c**, Representative images at different imaging rounds. The maximum exposure of 60 *z* planes of the same position in the tissue is displayed. Scale bar, 30 μm. **d**, Schematic representation of the root tip and UMAPs displaying root tip scRNA-seq data[18] used in this study. In the UMAPs, cells are labeled with cell types (left) and regions (right). LRC, lateral root cap; QC, quiescent center. **e**,**f**, Representative results from the imaging rounds 2 (**e**) and 3 (**f**). Left, UMAPs showing expression patterns of target genes. The colors of the gene name labels correspond to the colors in the images below. Middle, 3D projections (upper) and optical sections (2D, lower) of whole-mount tissue images. Right, representative cross-section views of the middle part of the samples (transition/elongation zone). Scale bar, 25 μm. Credit: *Nature Plants* (2023). DOI: 10.1038/s41477-023-01439-4

The cellular life inside a plant is as vibrant as the blossom. In each plant tissue—from root tip to leaf tip—there are hundreds of cell types that relay information about functional needs and environmental changes. Now, a new technology developed by Salk scientists can capture this internal plant world at an unprecedented resolution, opening the door for understanding how plants respond to a changing climate and leading to more resilient crops.

The method, called PHYTOMap, can capture entire plant tissues (like the whole root tip), instead of a small slice and provides insight into the complex biological conversations between cells that is difficult in two dimensions.

The method was detailed in *Nature Plants* on June 12, 2023, and the researchers expect PHYTOMap to be quickly popularized by the global

scientific community.

"PHYTOMap allows us to examine dozens of plant genes and see which cells express those genes, how cells influence each other, and how tissue architecture influences those cells," says Salk Professor Joseph Ecker, director of the Genomic Analysis Laboratory and Howard Hughes Medical Institute investigator. "We can then use those answers to improve crops, predict plant reactions to climate change, and more."

Existing imaging techniques can only view a small number of genes in one type of plant tissue and requires altering the plants' genetic makeup (creating transgenic lines). PHYTOMap (short for plant hybridization-based targeted observation of gene expression map) allows researchers to study dozens of genes simultaneously without any time-consuming genetic manipulation of the plant.

"PHYTOMap was able to map various cell-type-specific genes in expected locations of root tips in 3D," says Tatsuya Nobori, a postdoctoral researcher in Ecker's lab. "Now, we can use PHYTOMap to ask more complex questions, like how do different cell types respond and react to each other and their environment?"

In addition to being powerful, PHYTOMap is also accessible—the technique used is relatively standard and the associated cost is relatively minimal.

"With PHYTOMap, we will be able to ask so many new biological questions. I can't wait to use the method to see how plants interact with surrounding microorganisms," says Nobori.

"PHYTOMap makes visualizing cells in plant tissues so much easier—no need to alter the plant's genetic makeup, no need to flag cells with colorful markers," says Ecker, who is also the Salk International Council

Chair in Genetics. "I'm excited to see how PHYTOMap propels efforts to understand plant gene regulation during normal development and under various environmental conditions as well as how it may inform the optimization of agriculture."

In the future, the Ecker lab will use PHYTOMap to better understand the regulation of cell populations in various plant tissues to eventually engineer crops that are more resilient to climate change.

More information: Tatsuya Nobori et al, Multiplexed single-cell 3D spatial gene expression analysis in plant tissue using PHYTOMap, *Nature Plants* (2023). DOI: 10.1038/s41477-023-01439-4

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