New imaging technique drives biological molecules into technicolor

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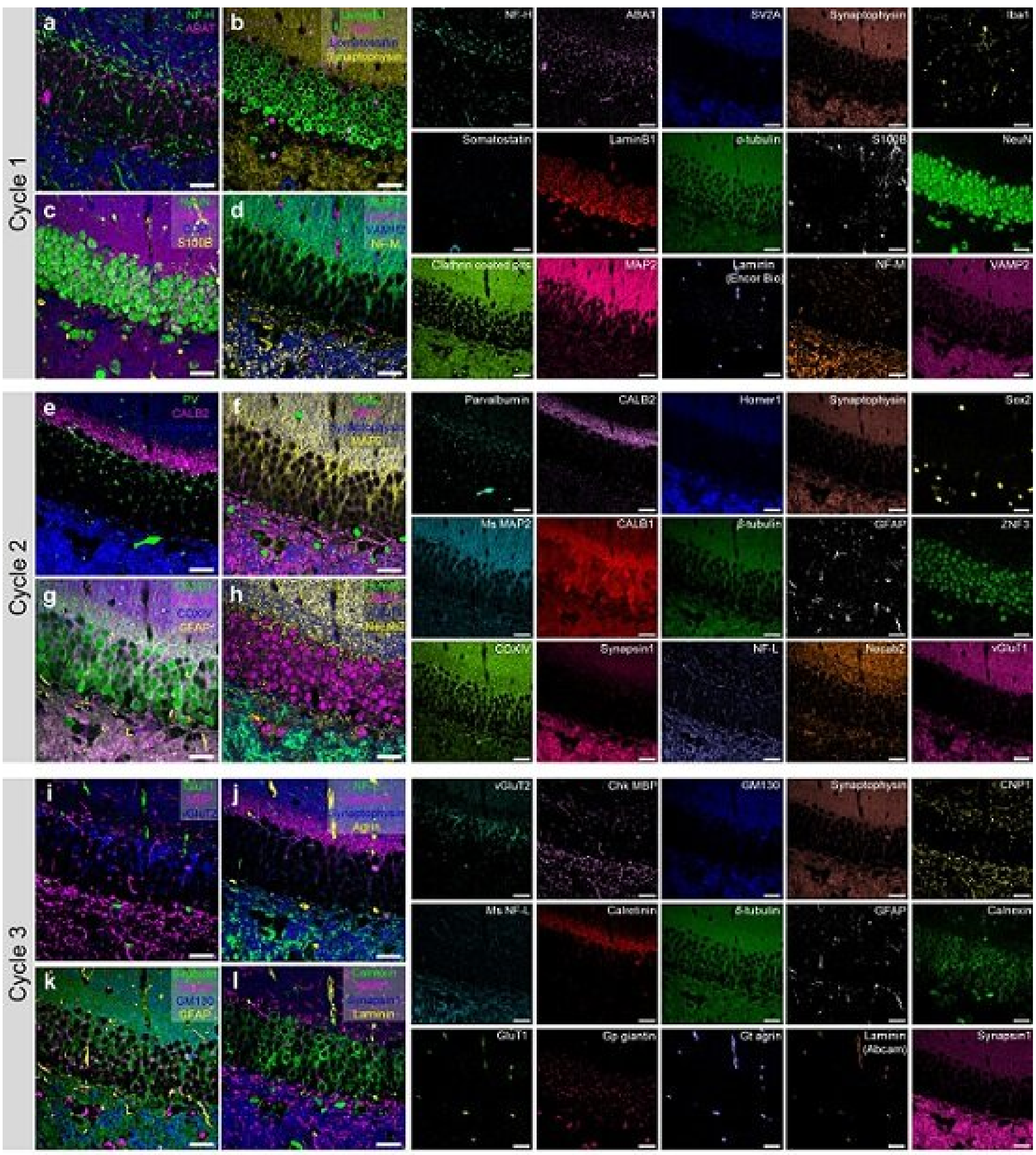

45-color multiplexed imaging of the mouse hippocampus via PICASSO in three staining and imaging rounds. Credit: *Nature Communications*/KAIST

Pablo Picasso's cubist artistic style shifted common features into unrecognizable scenes, but a new imaging approach bearing his namesake may elucidate the most complicated subject: the brain. Employing artificial intelligence to clarify spectral color blending of tiny molecules used to stain specific proteins and other items of research interest, the PICASSO technique, allows researchers to use more than 15 colors to image and parse our overlapping proteins.

The PICASSO developers, based in Korea, published their approach on May 5 in *Nature Communications*.

Fluorophores—the staining molecules—emit specific colors when excited by a light, but if more than four fluorophores are used, their emitted colors overlap and blend. Researchers previously developed techniques to correct this spectral overlap by precisely defining the matrix of mixed and unmixed images. This measurement depends on reference spectra, found by identifying clear images of only one fluorophore-stained specimen or of multiple, identically prepared specimens that only contain a single fluorophore each.

"Such reference spectra measurement could be complicated to perform in highly heterogeneous specimens, such as the brain, due to the highly varied emission spectra of fluorophores depending on the subregions from which the spectra were measured," said co-corresponding author Young-Gyu Yoon, professor in the School of Electrical Engineering at KAIST. He explained that the subregions would each need their own spectra reference measurements, making for an inefficient, timeconsuming process. "To address this problem, we developed an approach that does not require reference spectra measurements."

The approach is the "Process of ultra-multiplexed Imaging of biomolecules viA the unmixing of the Signals of Spectrally Overlapping fluorophores," also known as PICASSO. Ultra-multiplexed imaging refers to visualizing the numerous individual components of a unit. Like a cinema multiplex in which each theater plays a different movie, each protein in a cell has a different role. By staining with fluorophores, researchers can begin to understand those roles.

"We devised a strategy based on information theory; unmixing is performed by iteratively minimizing the mutual information between mixed images," said co-corresponding author Jae-Byum Chang, professor in the Department of Materials Science and Engineering, KAIST. "This allows us to get away with the assumption that the spatial distribution of different proteins is mutually exclusive and enables accurate information unmixing."

To demonstrate PICASSO's capabilities, the researchers applied the technique to imaging a mouse brain. With a single round of staining, they performed 15-color multiplexed imaging of a mouse brain. Although small, mouse brains are still complex, multifaceted organs that can take significant resources to map. According to the researchers, PICASSO can improve the capabilities of other imaging techniques and allow for the use of even more fluorophore colors.

Using one such imaging technique in combination with PICASSO, the team achieved 45-color multiplexed imaging of the mouse brain in only three staining and imaging cycles, according to Yoon.

"PICASSO is a versatile tool for the multiplexed biomolecule imaging of cultured cells, tissue slices and clinical specimens," Chang said. "We anticipate that PICASSO will be useful for a broad range of applications for which biomolecules' spatial information is important. One such application the tool would be useful for is revealing the cellular heterogeneities of tumor microenvironments, especially the heterogeneous populations of immune cells, which are closely related to cancer prognoses and the efficacy of cancer therapies."

More information: Junyoung Seo et al, PICASSO allows ultra-multiplexed fluorescence imaging of spatially overlapping proteins without reference spectra measurements, *Nature Communications* (2022). DOI: 10.1038/s41467-022-30168-z

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