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IMAGING

Off-the-shelf super-resolution microscopy in whole cells

The combination of DNA-PAINT and spinning-disk confocal microscopy makes super-resolution microscopy in whole cells affordable and easy to implement.

Super-resolution microscopy techniques have started to transform biological research in many areas. But these methods "are either hard to implement from the reagent side or complex to implement from the equipment side," explains Ralf Jungmann from the Ludwig Maximilian University Munich and the Max Planck Institute of Biochemistry in Martinsried, Germany. He and his colleagues published a combination of spinning-disk confocal (SDC) microscopy and DNA points accumulation for imaging in nanoscale topography (DNA-PAINT), which makes super-resolution microscopy easy to implement with commonly available microscopes.

Single-molecule localization microscopy (SMLM) methods such as stochastic optical reconstruction microscopy (STORM) and photoactivated localization microscopy (PALM) offer low-nanometer resolution and rely on stochastic switching of fixed and target-bound fluorescent labels. In contrast, PAINT-based methods use freely diffusing probes that bind statically or transiently to the target of interest.

A high signal-to-noise ratio (SNR) is crucial in SMLM for the detection of single-molecule fluorescent events. Selective plane-illumination approaches such as total internal reflection (TIRF) microscopy provide high SNR, but have limited penetration depths. As an alternative to TIRF microscopy, confocal microscopes offer optical sectioning and high SNR by blocking out-of-focus light using a pinhole. SDC microscopes are a variant of confocal microscopes that contain a spinning disk with an array of pinholes. They combine the advantage of high SNR with high-speed acquisition and the use of cameras as spatial detectors.

SDC microscopes have already been applied to perform SMLM experiments using PALM

EPIGENETICS

HUMANIZED YEAST—ERASING 1.3 BILLION YEARS OF EVOLUTION

Cellular engineering that allows budding yeast to survive with the four core human histones opens the door to exploring the function of histone variants and their modifications.

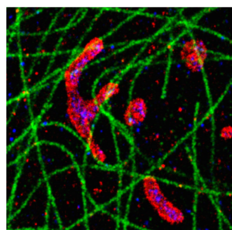
The original idea was simple, according to Jef Boeke from New York University Langone Health, who asked, "Can you swap out the yeast histone genes and replace them for human ones?"

Few genes in eukaryotes are as well conserved as the four core histones: H2A, H2B, H3 and H4, which make up the cylindrical structure of the nucleosome that is essential for packaging DNA. While yeast cells make do with only the four core histones, higher eukaryotes have evolved many more variants and additional modifications. Boeke's question was whether the human core histones, without the human machinery to deposit them on DNA, can function in the nucleus of budding yeast.

As straightforward as the question sounds, it has stumped researchers in the past.

Back in 2008 Junbiao Dai, who at the time was a postdoc in the Boeke lab, tried swapping human for yeast histones. He had just completed building a synthetic library in which every amino acid in histones H3 and H4 had been mutated. But, as Boeke recalls, the human for yeast histone swap did not work. Boeke blamed their lack of success on yeast's excellent ability to recombine and get rid of sequences it does not want.

Years later David Truong, a current postdoc in the Boeke lab, revisited the idea. Aside from understanding basic histone function, his ultimate goal is to build artificial human chromosomes in yeast, and for this he needs human chromatin and thus nucleosomes made up of human histones. He removed the histone genes from the yeast genome and supplied the human counterparts on a plasmid also carrying a positive selection marker. On another plasmid he provided the yeast histones, to ensure the initial survival of the



SDC-PAINT image of Alpha-Tubulin (green), TOM20 (red), and HSP60 (blue). Image adapted with permission from Schueder *et al.* (Springer Nature).

and STORM. However, excitation intensity and detection efficiency are suboptimal in SDC microscopy, which affects the achievable resolution and image quality in SMLM experiments. These "difficulties are not a problem for the DNA-PAINT technique, because the switching between bright and dark [states] is mediated by DNA hybridization reactions," Jungmann mentions. This decoupling of "switching" from the photophysical properties of the dye molecules, "combined with the very bright and photostable organic dyes that can be used for [DNA-PAINT], basically allowed us to implement [DNA-PAINT] on confocal hardware," Ralf Jungmann continues.

After evaluating the resolution of their system with DNA origami structures, the researchers demonstrated the capabilities of SDC microscopy in combination with DNA-PAINT (SDC-PAINT) on a range of biological targets. The researchers showed that SDC-PAINT is capable of resolving filamentous microtubule structures alongside the outer membrane and inner matrix in mitochondria throughout the whole cell.

To enable 3D super-resolution imaging with SDC-PAINT, the one modification necessary to their off-the-shelf SDC microscope "was using a cylindrical lens in front of the camera to give us optical astigmatism for point-spread-function engineering [to allow] three-dimensional [super]-resolution imaging," says Ralf Jungmann. The point-spread-function shaping allowed the researchers to visualize proteins located at the outer mitochondrial membrane and in the mitochondrial matrix. Finally, the authors demonstrate the versatility of SDC-PAINT by visualizing not only proteins, but also RNA and DNA.

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Schueder, F. *et al.* Multiplexed 3D super-resolution imaging of whole cells using spinning disk confocal microscopy and DNA-PAINT. *Nat. Commun.* **8**, 2090 (2017).

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cells, together with a strong negative selection marker. The combination of positive and negative selection should induce the yeast to eventually get rid of the yeast histones and exclusively use the human versions.

Like those who tried this approach before him, Truong noticed the challenge that "yeast hated human histones" and would use any homologous sequence to recombine and reacquire their own histones from the plasmid, leading to high background. "I had to eliminate any possibility that yeast histones have any sequence to recombine with," said Truong. Then all he could do was wait "for one yeast in 100 million to acquire the ability to become human." And the waiting paid off—after 10 to 18 days, he started to see small colonies that grew with human histones.

Whole-genome sequencing of the few clones revealed that additional mutations were needed, in cell-cycle checkpoint genes, but surprisingly never in the histone genes themselves. Systematic swapping of the histone residues from human back to yeast showed that retention of a handful of yeast residues in the N- and C-termini of H3 and H2A also helped. These optimizations led to faster growing yeast colonies with their entire genome packaged around human histones. Interestingly, the nucleosome spacing remained yeast like; and, while the cells grew in optimal medium, they had a hard time adapting to challenges such as a change in growth medium.

Histones in mammalian cells are heavily modified, but some equivalent residues lack modification in yeast histones. Swapping histones between the species will also elucidate the importance of such modifications. Truong also wants to introduce human genomic DNA sequences and explore the structural composition of human chromatin and human DNA in yeast.

And Boeke is not satisfied with stopping at nucleosomes. His question is to what extent they can humanize any pathway or structure in a yeast cell.

Nicole Rusk

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Truong, D.M. & Boeke, J.D. Resetting the yeast epigenome with human nucleosomes. *Cell* **171**, 1508–1519 (2017).