DNA-PAINT super-resolution microscopy at speed

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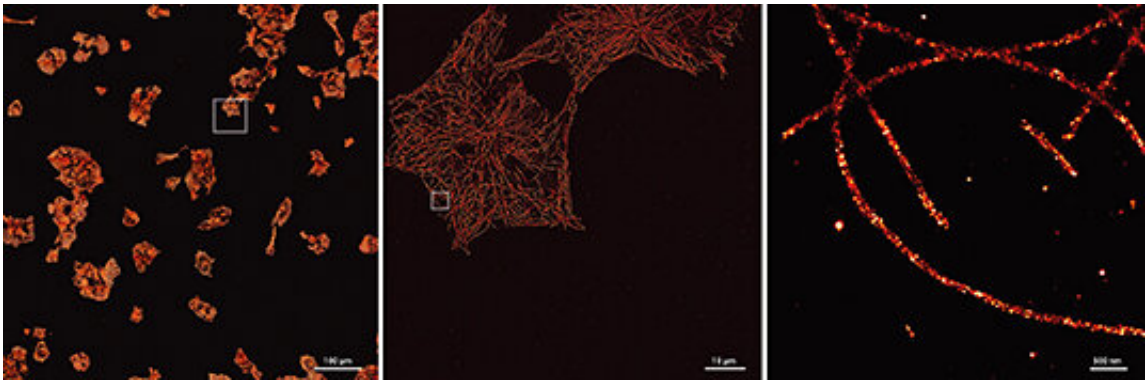

Super-resolved DNA-PAINT allows the detection of the ultrastructure of cells, such as microtubules, which are part of the cytoskeleton. Credit: Florian Schueder, MPI of Biochemistry

Recent advances in fluorescence microscopy allow researchers to study biological processes below the classical diffraction limit of light. Ralf Jungmann, Professor for Experimental Physics at Ludwig-Maximilians-Universität in Munich and research group leader at the Max Planck Institute of Biochemistry, and colleagues developed DNA-PAINT, a variant of these so-called super-resolution approaches. "DNA-PAINT yields super-resolved images using comparably simple microscopes", says Jungmann. The technique uses short, dye-labeled DNA strands that transiently interact with their target-bound complements in order to create the necessary "blinking" for super-resolution reconstruction. This approach enables sub-10-nm spatial resolution and easy multiplexing

through the use of orthogonal DNA sequences for different targets.

"In recent years, we have optimized DNA-PAINT in a few key areas. However, one major limitation still persists, which prevents DNA-PAINT to be applied to biomedically relevant high-throughput studies: The rather slow image acquisition speed", says Jungmann. Classical DNA-PAINT experiments can easily last from tens of minutes to hours. "We have checked carefully why this takes so long", says Florian Schüder, lead author of the current study and co-worker in Jungmann's group. "Optimized DNA sequence design and improved image buffer conditions allowed us to speed things up by an order of magnitude", adds Schüder.

From the DNA origami breadboard to cells

In order to quantitatively assess the improvements to DNA-PAINT, the researchers used DNA origami structures, which are self-assembled, nanometer-sized DNA objects autonomously folding into predefined shapes. These structures can be used to arrange DNA-PAINT binding sites spaced precisely at e.g. 5-nm distances. This allowed the researchers to evaluate the speed improvement in DNA-PAINT using well-defined conditions. In a next step, the team applied the speed improvement also to a cellular system. For this, microtubules, which are part of the cytoskeleton, were visualized at super-resolution, 10-times faster than before. "The increased imaging speed allowed us to acquire an area of one square millimeter at a resolution of 20 nm in only 8 hours. This would have taken us almost four days before", explains Schüder.

Ralf Jungmann concludes: "With these current improvements, which allow us to image 10-times faster, we bring DNA-PAINT to the next level. It should now be feasible to apply it to high-throughput studies with biological and biomedical relevance e.g. in diagnostic applications."

More information: Florian Schueder et al. An order of magnitude faster DNA-PAINT imaging by optimized sequence design and buffer conditions, *Nature Methods* (2019). DOI: 10.1038/s41592-019-0584-7