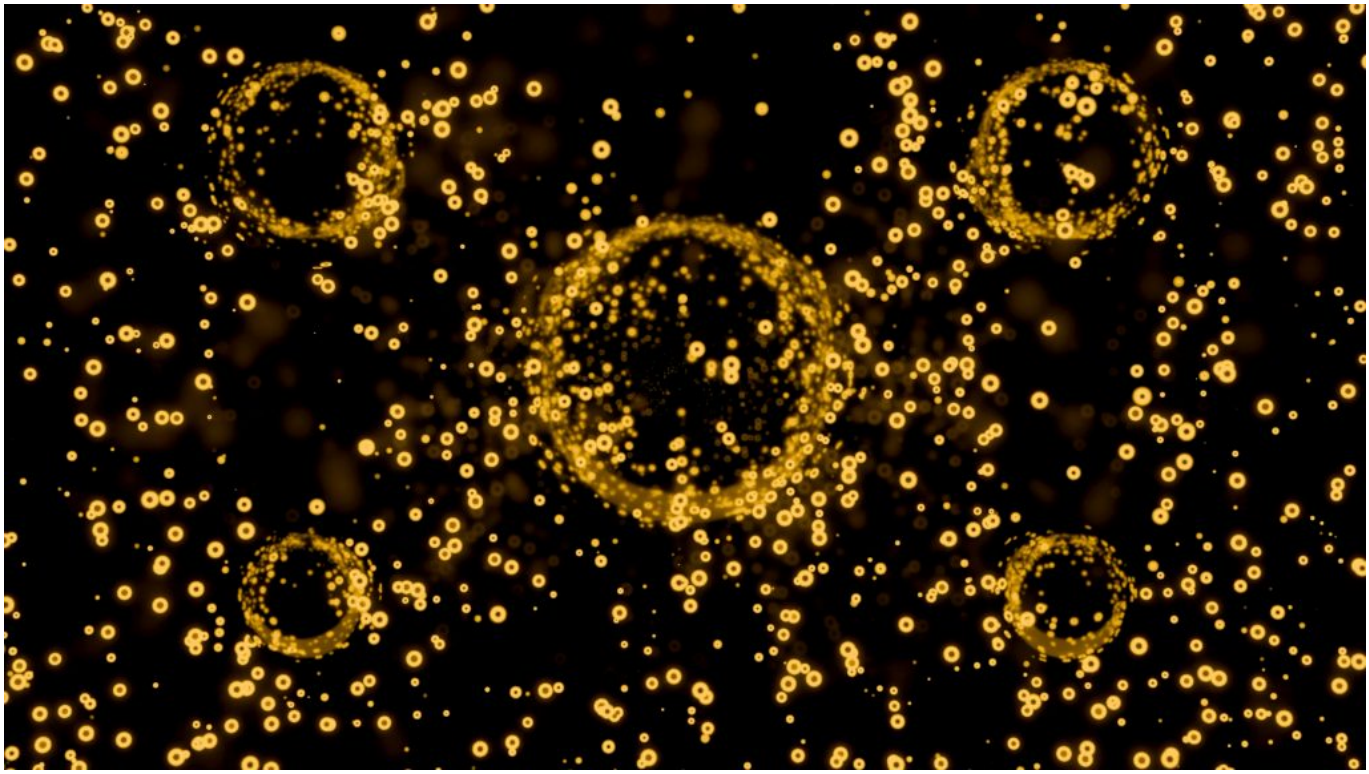
First reported in 2010, DNA points accumulation for imaging in nanoscale topography (DNA-PAINT) is a super-resolution microscopy (SRM) technique that can be performed with existing antibody reagents and standard instrumentation – features that have led to its use by a growing number of researchers. Here, we explain the underlying principles of DNA-PAINT and look at how the original method has been improved to address various imaging challenges.



DNA-PAINT and super-resolution microscopy

To understand how DNA-PAINT fits within the broader context of super-resolution microscopy, it is important to remember that SRM techniques break through the optical resolution limit of fluorescence microscopy by either using patterned illumination to reduce the size of the point spread function or employing single-molecule localization. DNA-PAINT is a single-molecule localization microscopy (SMLM) technique, based on transient binding between complementary single-stranded DNA (ssDNA) oligonucleotides. Its mechanism of action distinguishes DNA-PAINT from other SMLM methods, such as stochastic optical reconstruction microscopy (STORM) and photoactivated localization microscopy (PALM), which instead depend on the stochastic photo-activation of fluorophores that are permanently bound to a target of interest.

Origins of DNA-PAINT

DNA-PAINT evolved from PAINT, a method first described in 2006 by Alexey Sharonov and Robin Hochstrasser1. Using the lipophilic stain, Nile Red, for repeated cycles of binding, imaging, and photobleaching, Sharonov and Hochstrasser were able to produce super-resolution images of large unilamellar vesicles. Further development of this method by Giannone *et al*. saw universal PAINT (uPAINT) reported in 2010, in which different fluorophore/ligand combinations are used for imaging specific biomolecules2. In parallel, Jungmann *et al.* developed DNA-PAINT as a means of resolving DNA nanostructures, subsequently extending their method to enable imaging of proteins in fixed cells3,4.

Principles of DNA-PAINT

DNA-PAINT is based on transient binding between two complementary ssDNA strands, typically 8-10 nucleotides in length. One strand, known as the docking strand, is linked to an antibody that recognizes a specific target. The other, known as the imager strand, is covalently bound to a fluorophore and diffuses freely in solution. When the two strands hybridize, an increase in fluorescence occurs until the imager strand is released. Factors influencing the duration of this fluorescent 'blink' include the DNA sequence, which determines the off-rate, and the concentration of the imager strand. Once many thousands of blinks have been captured, SMLM algorithms are used to deconvolute the data. Compared with techniques such as STORM and PALM, DNA-PAINT is not restricted by photobleaching, since the fluorophores are continually replenished.

A rapidly evolving technology

Despite being easy to implement, DNA-PAINT is hampered by high background and slow imaging speeds, partly due to the presence of unbound fluorophores in solution. To address these problems, as well as increase the overall scope of DNA-PAINT, the original method has been adapted to form techniques including the following:

Exchange-PAINT (2014)

Building on their successful development of DNA-PAINT, Jungmann *et al*. established a multiplexed approach based on sequential imaging of different targets with the same fluorophore4. Initially, the targets are labeled with distinct docking strands, then the first imager strand is added and an image is captured. Next, the first imager strand is washed away and the process is repeated with additional imager strands. Using this method, Jungmann *et al*. were able to generate 10-plex super-resolution images for DNA structures and 4-plex super-resolution images for fixed HeLa cells.

q-PAINT (2016)

Also developed by Jungmann *et al*., quantitative PAINT (qPAINT) uses the predictable binding kinetics between docking and imager strands to quantify the number of targets without spatially resolving them5. Importantly, qPAINT improves the counting accuracy compared to spatial counting, which can under-estimate the total number of proteins due to resolution-based imaging challenges.

FRET-PAINT (2017)

Förster resonance energy transfer (FRET) occurs when two fluorescent molecules (a donor and an acceptor) are in close proximity to one another. Following excitation of the donor, energy is transferred to the acceptor, causing it to emit a fluorescent signal. By using donor-labeled imaging strands for DNA-PAINT and detecting only the acceptor fluorescence, Auer *et al.* were able to significantly increase the concentration of the imaging strands while also reducing background fluorescence, enabling super-resolution images of microtubules to be captured in under 30 seconds6.

PRISM (2019)

Probe-based imaging for sequential multiplexing (PRISM), developed by Guo *et al*., uses imaging strands with different binding affinities for the same docking strand partner7. It involves incubating the sample with docking strand-conjugated antibodies for multiple targets, before sequentially adding imager strands that are specific to each marker. Confocal microscopy images are obtained using high affinity imager strands, while super-resolution microscopy images are obtained using low-affinity imager strands that have a modified sequence.

DNA-PAINT-ERS (2020)

The addition of ‘ERS’ to the original DNA-PAINT name denotes three protocol modifications that improve DNA hybridization kinetics8. ‘E’ refers to the addition of ethylene carbonate to the imaging buffer, ‘R’ indicates the introduction of sequence repeats to the docking strand, and ‘S’ covers the inclusion of a spacer between the docking strand and the affinity agent. By accelerating the process of docking strand-imager strand unbinding, DNA-PAINT-ERS provides faster imaging and improved resolution compared to DNA-PAINT.

Check out the publication to see how Jackson ImmunoResearch secondary antibodies were used during the development of DNA-PAINT-ERS.

LIVE-PAINT (2020)

Instead of exploiting the transient binding of complementary DNA strands, live cell imaging using reversible interactions-PAINT (LIVE-PAINT) relies on reversible peptide-protein interactions9. Specifically, a short peptide sequence is fused to the protein being studied, which is expressed from its endogenous promoter, while a peptide-binding protein is fused to a fluorescent protein, which is expressed from an inducible promoter. A main advantage of this method is that it can be used for imaging in live cells. In their proof-of-concept studies, Oi *et al.* were able to track multiple protein targets inside living *S. cerevisiae*.

Repeat DNA-PAINT (2021)

Developed by Clowsley *et al*. as a means of reducing the imager probe concentration, which is known to contribute to high background signal, Repeat DNA-PAINT uses docking strands with identical repeat domains that can bind to multiple imagers10. Validation of this approach included testing with optically thick tissue samples, which are notorious for high background, and demonstrated a 5-fold reduction in free-imager background, equating to 6-10x faster data acquisition.

Our AffiniPure™ Goat Anti-Mouse IgG (H+L) facilitated the development of Repeat DNA-PAINT – learn more in the publication.

Fluorogenic DNA-PAINT (2022)

A defining feature of fluorogenic DNA-PAINT is its use of imager probes that are conjugated to both a fluorophore and a quencher, such that they do not fluoresce when free in solution11. In addition, the docking strands include internal mismatches to facilitate faster off-rates. These adaptations have resulted in a 57-fold increase in fluorescence over regular DNA-PAINT, eliminating the need for optical sectioning when imaging whole cells in 3D, as well as provided a 26-fold increase in imaging speed. Fluorogenic DNA-PAINT also allows for simultaneous imaging in separate color channels with two or more probe combinations.

Discover how Jackson ImmunoResearch secondary antibodies were used to develop fluorogenic DNA-PAINT in our literature review.

DNA-PAINT MINFLUX Nanoscopy (2022)

Minimal fluorescence photon fluxes (MINFLUX) nanoscopy is an imaging technique that uses a doughnut-shaped excitation beam to illuminate photoswitchable fluorophores, with coordinate targeting being applied to minimize the number of photons required for localization12. To overcome the constraints of using photoswitchable fluorophores for MINFLUX, which include having to match the brightness and switching kinetics of different fluorophores within a narrow range, Ostersehlt *et al.* combined MINFLUX with sequentially multiplexed DNA-PAINT, which facilitated 3-color imaging of mitochondria in mammalian cells13.

Dual-color DNA-PAINT single-particle tracking (2023)

Single-particle tracking (SPT) methods are widely used for observing molecular behaviors and interactions in live cells. However, experimental accuracy hinges on label brightness and photostability, as well as requires that fluorophores be conjugated with their target molecules in a one-to-one stoichiometry. DNA-PAINT based single-particle tracking (DNA-PAINT-SPT), developed by Niederauer *et al.*, allows for tracking single particles over an extended period of time without concerns over photobleaching, and has proven utility for dual-color SPT in living cells14.

SUM-PAINT (2024)

Secondary label-based unlimited multiplexed DNA-PAINT (SUM-PAINT) is a method that decouples DNA barcoding of the target from the imaging process to accelerate label exchange and image acquisition15. First, target-specific antibodies are incubated with VHH fragment (nanobody) format secondary antibodies that each carry a unique DNA barcode, then transient adaptors are added (each including a barcode complement sequence and a speed-optimized DNA-PAINT docking sequence). Following this, fluorophore-labeled, speed-optimized imager strands are introduced to enable target visualization. With SUM-PAINT, Unterauer *et al.* were able to generate 30-plex datasets in neurons, which revealed a distinct synapse type.

Download the publication to see how Jackson ImmunoResearch secondary antibodies were used in the development of SUM-PAINT.

FLASH-PAINT (2024)

Fluorogenic labeling in conjunction with transient adapter-mediated switching for high throughput DNA-PAINT (FLASH-PAINT) is, as the name suggests, another method based on the use of transient adaptors16. However, the adaptors are added at the same time as the imager strands, and are removed with an eraser following image capture. Using FLASH-PAINT, Schueder *et al*. performed a variety of applications, including 9-target imaging of primary cilia and monitoring changes in the proximity of 13 different targets in unperturbed and dissociated Golgi stacks.

Conclusion

DNA-PAINT and its related methods offer researchers an easy way of performing super-resolution microscopy with standard instrumentation and commercially available antibody reagents, such as secondary antibodies from Jackson ImmunoResearch. As further improvements to the original method are made, offering faster image acquisition, reduced background signal, and improved resolution, the use of DNA-PAINT looks set to increase further.

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