

FASTER DNA-PAINT IMAGING BY OPTIMIZED DNA SEQUENCE DESIGN

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Advances in optical super-resolution techniques make it possible to image biological processes below the classical diffraction limit of light. DNA Points Accumulation in Nanoscale Topography (DNA-PAINT) is a simple implementation of single-molecule localization microscopy with spatial resolution better than 5 nm demonstrated on artificial DNA nanostructures [1]. However, image acquisition times are relatively slow compared to other approaches such as STED and STORM. In fact, the imaging speed is limited by the DNA hybridization kinetics of the fluorophore labeled imager strands in solution and the docking strand DNA on the target molecule.

The hybridization efficiency has been successfully improved by an order of magnitude using a two-letter code sequence design and optimized buffer conditions [2]. Despite the significant speed improvement, this design is limited to one color imaging due to lack of orthogonal sequences. To overcome this limitation and further increase the DNA binding kinetics, we use concatenated sequences with overlapping binding sites. We were able to speed-up hybridization kinetics by another order of magnitude and show fast 6 color multiplexed imaging without sacrificing spatial resolution.

To implement this approach in cells, we site-specifically and quantitatively conjugate DNA docking sites to nanobodies and image 4 different receptor tyrosine kinase proteins with single molecule resolution (below 10 nm) in the same cell.

Overall, optimized DNA-PAINT sequence design does not only improve the imaging speed but also specificity, sensitivity and spatial resolution particularly for cellular imaging, hence making it a promising approach to a broader range of biological applications.

References

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