Fluorogenic probe for fast 3D whole-cell DNA-PAINT

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Conventional DNA-PAINT microscopy can achieve better than 20 nm resolution and, unlike STORM and PALM, is resistant to photobleaching and the blinking kinetics are tunable independently of the fluorophore. However, there are two severe limitations with current implementations: i) it is very slow, taking hours to days for a single image, and ii) some method of optical sectioning is necessary, i.e. it is largely incompatible with widefield illumination.

We created a novel fluorogenic imager probe based on a fluorophore-quencher design. On binding to its complementary sequence, probe fluorescence increases by 77 fold. To optimize for fast blinking kinetics, the probe was paired to a docking sequence with partial mismatches to decrease binding affinity.

Reduced background fluorescence with the fluorogenic probe allowed imaging at significantly higher framerates (100 Hz) compared to conventional DNA-PAINT. We could resolve the walls of microtubules in fixed COS-7 cells within minutes, with an approximately 26× faster rate of blinking.

Furthermore, it is now possible to perform DNA-PAINT under widefield illumination. We demonstrated this by combining DNA-PAINT with 4Pi SMS microscopy to image mitochondria in 3D. The synergy between photobleaching resistance and moderate imaging rate permits the collection of super-resolution volumes at a quality and sampling-density that has not been previously achievable.

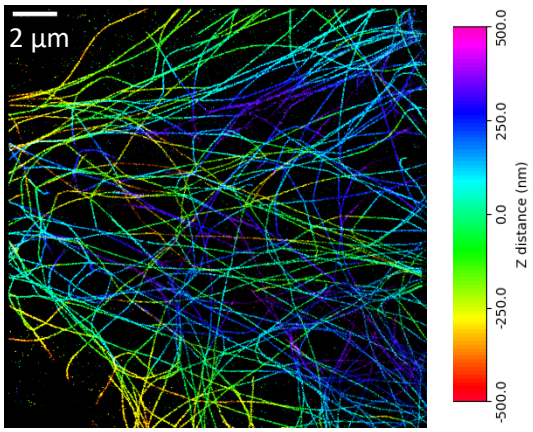

Figure 1:
3D DNA-PAINT imaging of microtubules at 100 Hz for 5 min.