FLAT-TOP TIRF ILLUMINATION BOOSTS DNA-PAINT IMAGING AND QUANTIFICATION

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Single-molecule super-resolution (SR) techniques have extended the spatial resolution of optical microscopy down to a few nanometers. However, quantitative treatment of SR data remains a difficult task due to its complex dependence on a manifold of experimental parameters. Among the different SR variants, DNA-PAINT (Point Accumulation for Imaging in Nanoscale Topography) is relatively straightforward to implement, since it achieves the necessary 'blinking' without the use of rather complex optical or chemical activation schemes. However, it still suffers from image and quantification artifacts caused by inhomogeneous optical excitation. Here we demonstrate that several experimental challenges can be alleviated by introducing a segment-wise analysis approach and ultimately overcome by implementing a flat-top illumination profile for total internal reflection fluorescence (TIRF) microscopy using a commercially-available beam-shaping device. The improvements with regard to homogeneous spatial resolution and precise kinetic information over the whole field-of-view were quantitatively assayed using established DNA origami and cell samples. Our findings now open the door to high-throughput studies using DNA-PAINT with thus far unprecedented accuracy for quantitative data interpretation.