Ratio-PAINT, an alternative to time multiplexed multi-colour DNA-PAINT.

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Visualisation of subcellular interactions at the molecular scale is achievable with multi-colour super-resolution microscopy techniques. The use of oligonucleotide nano-technologies implemented in DNA-PAINT[1] routinely achieve nanometre localisation precision[2] with little change to the method of labelling or detection. Current DNA-PAINT based multi-colour approaches generally utilise a time-multiplexed sequential imaging protocol such as Exchange-PAINT[1] or Quencher-Exchange-PAINT[3]. In these techniques 'imager' strands are removed via buffer exchanges or with the introduction of a competing 'quencher' strand actively targeting its complementary imager. The subsequent imager is orthogonal to the prior design and transiently binds to its designated target. Here, we present an alternative to time-multiplexed multi-colour DNA-PAINT by using ratiometric dual-channel imaging to distinguish between different imager strands modified with distinct dyes. An optical image splitter with computational nanoscale correction of chromatic aberrations is used to split the colours. This method, termed Ratio-PAINT, considerably accelerates the acquisition of multi-colour super-resolution imaging, removes the risk of sample displacement or the need for fluid replacement mechanisms and therefore minimise contributions to drift. We conduct Ratio-PAINT to image optically thick biological tissue and further discuss the merits of our technique.

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