DNA-PAINT IN TISSUE SAMPLES AND USE OF FLUORESCENCE QUENCHERS FOR RAPID IMAGER SWITCHING

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Utilising the properties and programmability of DNA molecules, the optical super-resolution imaging technique DNA-PAINT allows quantitative, multiplexed and 3D imaging of cellular structures at single nanometre resolution. The transient binding of short fluorescently labelled oligonucleotides (imager strands) to immobilised docking strands in the sample results in an apparent blinking enabling localisation based super-resolution. The solution exchange of imager strands underlies Exchange-PAINT which enables multiplexed imaging that avoids chromatic aberrations [1]. However, in thick samples, such as biological tissue sections, diffusional exchange of imager strands is slow.

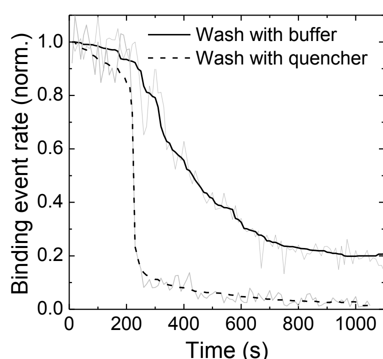

Figure 1: Reducing effective imager strand concentration by washing with plain buffer or with added fluorescence quenchers.

We demonstrate the adaptation of DNA-PAINT protocols to tissue imaging which enabled high-quality super-resolution imaging of membrane structures and protein clusters. In these samples, diffusional imager wash-out takes minutes even if the surrounding buffer solution is completely replaced. To overcome the comparatively slow imager strand switching, we developed a new approach using quencher strands. The use of fluorescence quenchers has previously been proposed to reduce background [2]. We found, both experimentally and using a model of DNA hybridisation, that while background levels are reduced with quencher strands the binding rates of imager to docking strands are reduced in direct proportion. In other words, the addition of quenchers is equivalent to a decrease of imager concentration. This can be used to accelerate Exchange-PAINT of tissue samples, where efficient strand exchange is required to minimise crosstalk. Quencher addition reduces unwanted imager concentrations to negligible levels much more rapidly (Fig. 1), and thus enables efficient multiplexed imaging in tissue samples.

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