Methods for correction of multiple-blinking artefacts and cluster analysis of SMLM data

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Photoactivated localisation microscopy (PALM) produces an array of localisation coordinates by means of photoactivatable fluorescent proteins. However, observations are subject to fluorophore multiple-blinking and each protein is included in the dataset an unknown number of times at different positions, due to localisation error. This causes artificial clustering to be observed in the data. We present a workflow using calibration-free estimation of blinking dynamics and model-based clustering, to produce a corrected set of localisation coordinates now representing the true underlying fluorophore locations with enhanced localisation precision. These can be reliably tested for spatial randomness or analysed by other clustering approaches, and previously inestimable descriptors such as the absolute number of fluorophores per cluster are now quantifiable, which we validate with simulated data and experimental data. We also present the latest cluster analysis methodologies from our lab, including machine-learning approaches. We apply these methods to study the organisation of proteins at the T cell immunological synapse.