

Spectrally red-shifted fluorescent fiducial markers for optimal drift correction in localization microscopy

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Single-molecule localization microscopy (SMLM) enables to unravel the molecular architecture of biological structures below the diffraction limit of light by spatiotemporally separating and localizing single molecules [1]. In general, SMLM image quality correlates with labeling density and number of localizations which necessitates long imaging schemes. However, sample drift caused by mechanical vibrations or thermal expansion increases over time and may be detrimental to image quality. Thus, precise drift correction is crucial for optimal SMLM results. One common approach for drift correction is the addition of fiducial markers that ideally are bright, photostable, have low intensity fluctuations and do not saturate the camera.

Here, we evaluate commonly used fiducial markers such as gold nanoparticles, fluorescent beads, and nanodiamonds. We show that gold nanoparticles and nanodiamonds exhibit a low signal emission which results in less precise drift correction, TetraSpeck microspheres display fluorescence signal fluctuations under SMLM imaging conditions which bias drift correction.

To circumvent these limitations, we introduce a novel concept that utilizes spectrally red-shifted fluorescent beads that are excited far distant from their excitation maximum as optimal fiducial markers. We demonstrate that this simple yet powerful concept can improve drift correction in structural PALM, sptPALM and DNA-PAINT experiments. Furthermore, we show spectrally red-shifted fluorescent beads are compatible with different imaging conditions in different spectral channels [2]. We conclude that drift correction with spectrally red-shifted fluorescent beads improves the quality of SMLM results.

[1] Turkowyd, B., D. Virant, and U. Endesfelder, *From single molecules to life: microscopy at the nanoscale*. Anal Bioanal Chem, 2016. **408**(25): p. 6885-911.

[2] Balinovic, A., et al., *Spectrally red-shifted fluorescent fiducial markers for optimal drift correction in localization microscopy*. J Phys D, 2019. *under revision*.