Enabling Spectrally Resolved Single-Molecule Localization Microscopy at High Emitter Densities in a 4f Geometry

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Abstract

Single-molecule localization microscopy (SMLM) is a powerful technique for elucidating structure and dynamics in the life- and material sciences with sub-50 nm spatial resolution. The simultaneous acquisition of spectral information (spectrally resolved SMLM, sSMLM) enables multiplexing using spectrally distinct fluorophores or enable the probing of local chemical environments with solvachromatic fluorophores such as Nile Red. Until recently, the widespread utilisation of sSMLM was hampered by several challenges: an increased complexity of the optical detection pathway, limited software solutions for data analysis, lower accessible emitter densities or smaller field-of-views, and overall reduced spatio-spectral resolution.

In our previous implementation, we placed a blazed, low-dispersion transmission grating positioned close to the camera (1). In this configuration, the +1st diffraction order was minimally elongated compared to the point spread function of the 0th order and could therefore be analysed using common sub-pixel single-molecule localization algorithms. Furthermore, the distance between both PSFs provided accurate information on the spectral properties of the emitter.

Here, we present new data using a grating that was placed next to the first image plane in a 4f detection geometry. In this updated configuration, the distance between the 0th and the 1st order can be better controlled further enabling the addition of additional optical elements such as cylindrical lenses to enable three-dimensional resolved sSMLM.

References

1. Martens, K. J. A.; Gobes, M.; Archontakis, E.; Brillas, R. R.; Zijlstra, N.; Albertazzi, L.; Hohlbein, J. Enabling Spectrally Resolved Single-Molecule Localization Microscopy at High Emitter Densities. Nano Lett. 2022, 22 (21), 8618–8625. https://doi.org/10.1021/acs.nanolett.2c03140.