ACHROMATIC LIGHT PATTERNING AND IMPROVED IMAGE RECONSTRUCTION FOR PARALLELIZED RESOLFT NANOSCOPY

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Fluorescence microscopy is rapidly turning into optical nanoscopy. Among the various nanoscopy methods, the STED/RESOLFT super-resolution family has recently been expanded to image larger fields of view within a few seconds [1–3]. This advance relies on using light patterns featuring substantial arrays of intensity minima for discerning features by switching their fluorophores between ‘on’ and ‘off’ states of fluorescence.

Here we exploit that splitting the light with a grating and recombining it in the focal plane of the objective lens renders arrays of minima with wavelength-independent periodicity [4]. This color-independent creation of periodic patterns facilitates coaligned on- and off-switching and readout with combinations chosen from a range of wavelengths. Applying up to three such periodic patterns on the switchable fluorescent proteins Dreiklang and rsCherryRev1.4, we demonstrate highly parallelized, multicolor RESOLFT nanoscopy in living cells at 60–80 nm FWHM and sub-100 nm resolution for up to about 100×100 μm^2 fields of view. We discuss the impact of novel image reconstruction algorithms featuring background rejection by spatial bandpass filtering, as well as strategies that incorporate complete image formation models.

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