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Background-Free Super-Resolution Microscopy of Subcellular Structures by Lifetime Tuning and Photons Separation

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dictable number, determined by the amount of noise. The method also isolates any uncorrelated background signal. We demonstrate that this spectroscopy-based method provides background-free nanoscale imaging of subcellular structures, opening new routes in super-resolution microscopy based on the encoding of spatial information through manipulation of molecular dynamics. We discuss advantages and limitations considering application of the method to the imaging of sparse cytoskeletal structures and large scale organization of chromatin.

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Background-Free Super-Resolution Microscopy of Subcellular Structures by Lifetime Tuning and Photons Separation

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The visualization at the nanoscale level inside cells is a fundamental need in molecular biology. The challenge of increasing the spatial resolution of an optical microscope beyond the diffraction limit can be reduced to a spectroscopy task by proper manipulation of the molecular states. The nanoscale spatial distribution of the molecules inside the detection volume of the microscope can be encoded within the fluorescence dynamics and can be decoded by resolving the signal into its dynamics components [1]. We present here a robust and general method, based on the phasor analysis [2], to spatially sort the fluorescent photons on the basis of the associated molecular dynamics and without making use of any fitting procedure. In a specific implementation of this method, we generate spatially controlled gradients in the fluorescence lifetime by stimulated emission [3]. The separation of the time-resolved fluorescence components sorts photons according to their spatial positions. Spatial resolution can be increased indefinitely by increasing the number of resolved components up to a maximum, pre-