RESOLUTION ENHANCEMENT IN NONLINEAR SCANNING MICROSCOPY THROUGH POST-DETECTION DIGITAL COMPUTATION

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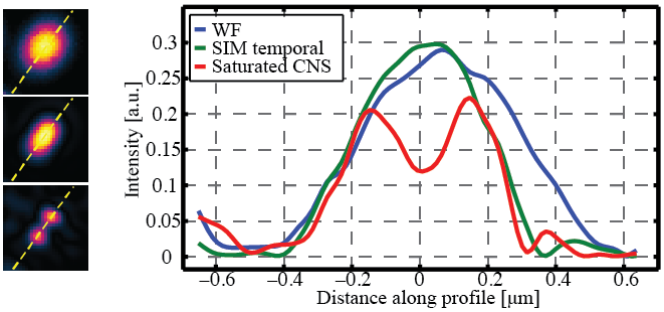
Confocal microscopy with a small pinhole improves the lateral resolution by a factor of √2 but at the cost of reduced signal intensity. This problem has been solved by removing the pinhole, using a camera detector in the imaging plane and reassigning each pixel signal to the most probable scanning position, as proposed by Sheppard [1]. However this technique relies on the fact that the excitation and collection Point Spread Functions (PSFs) are identical in shape and the resolution enhancement is inherently limited under two times. We demonstrate here thata different post-detection digital data processing method [2] based on Fourier filteringcan overcome this limitation. This computational post treatment allows the combination of camera based scanning microscopy with nonlinear effects that increase the frequency content but modify the shape of the effective excitation PSF.



We present an experimental resolution enhancement of scanning microscopy by a factor of 2.5 times over the classical resolution limit, by the combination of fluorescence saturation and digital post treatment [3]. We named this techniqueComputational Nonlinear Scanning (CNS) microscopy. The obtained experimental resolution improvement, quantified by imaging of 40nm fluorescent non-bleaching nanocrystals is surprisingly low compared to other fluorescence saturation based technique like saturated Structured Illumination Microscopy (SIM). We propose a simple analysis of the impact of photon detection noise the resolution performance of different type of microscopy. We show that CNS microcopy with a focused spot leads to a worst signal to noise ratio performance than saturated SIM and we propose different excitation patterns to improve the performance of the technique over the high frequencies of the optical transfer function.

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